

Where next with psychiatric illness?

New research has shown some schizophrenia to be, in part, genetically determined, which promises new insight into psychiatric illness. But the need for enlightened social policies remains urgent.

SCHIZOPHRENIA is arguably the worst disease affecting mankind, even AIDS not excepted. For schizophrenia, a form of madness, is the means by which more than one in 1,000 people are disabled early in life, often in their late teens, and as a consequence are transformed from people capable of contributing to the well-being of society into people who are, at best, dependent on society and often disruptive of it. The emotional damage done by schizophrenia, not merely to those who suffer from it, but to their families and friends, has been thoroughly documented in the past two decades. By comparison, AIDS, while also a means by which people are disabled much earlier in life than they would normally expect to be overtaken by the unavoidable diseases of natural mortality, is for the time being an order of magnitude less common even in those communities in which it is established. And while the pathos of AIDS rests on the fact that a large proportion of those affected die, how is that tragedy rationally to be compared with the circumstance that a large proportion of those suffering from schizophrenia must limp through a normal lifespan incapable of functioning as normal people?

Indeed, schizophrenia is the more daunting because so little is known of its causation. While arguments may persist that infection by the virus HIV (human immunodeficiency virus) is not a sufficient explanation of the transmission of AIDS, at least enough is known for rational avoidance strategies to be possible. The occurrence of schizophrenia, by contrast, seems mostly a bolt from the blue for those affected. Even the diligent practice of what has been painfully learned in half a century about the proper bringing-up of children gives no assurance that schizophrenia will not strike. And while much has been learned about the treatment of other forms of psychiatric illness, manic depression, for example, schizophrenia remains obdurately susceptible only to palliation. Since the early 1960s, the use of phenothiazine drugs has made it possible for the majority of people with schizophrenia lucky enough to live in prosperous communities to be returned from incarceration into the community, but at the cost of the lifelong administration of drugs whose long-term side-effects are not fully known, in the process presenting even the most advanced societies with challenges still not met.

How will these sad circumstances be changed by the research now reported from the University College and Middlesex School of Medicine (page 164) and the Yale University School of Medicine (page 167)? A great deal, but not definitively. That is the burden of the perceptive and judicious comment by Dr Eric Lander on page 105.

Genetic defect?

The London group has demonstrated that cases of schizophrenia among some Icelandic families are linked with genetic defects of an unknown gene located somewhere on chromosome 5. Those carrying the gene do not invariably develop the disease, but are merely susceptible to it. Even so, the study suggests that the unidentified gene is dominant; those carrying a single copy of it inherit the susceptibility. But the study does not show that all schizophrenia arises from this or indeed from any other genetic defect. Indeed, the Yale group provides a counter-example — a Swedish family in which cases of schizophrenia are not linked

with the defect on chromosome 5. Moreover, the Iceland study does not show that the defect, not yet identified, on chromosome 5, is a sufficient cause of schizophrenia; maybe other unknown conditions must be satisfied before the disease develops.

With all those reservations, the new development is a most important stepping-stone for further research that must throw more light on the causation of schizophrenia. When the gene concerned has been located exactly, and perhaps its nucleotide sequence determined, it will also be possible to embark on studies of the mechanism of the disease, presumably biochemical in character, that may (with luck) be more generally applicable. Then it will be feasible to seek means of designing drugs or other forms of treatment which are more specific and more effective than those available. Again, it will be reasonable to hope that the outcome of that research will be more generally applicable than only to patients inheriting a susceptibility to schizophrenia through a defective gene on chromosome 5. This, it will be recognized, is not a recipe for quick results, but the outline of a programme of research spanning decades. Yet the prospect now offered of a systematic route to an understanding of the mechanism of schizophrenia is a great advance.

Familial studies

Lander explains, among many other things, how the studies now reported should be a further spur to the genetic approach to the understanding of psychiatric disease. In the past few years, with the development of the technique for using restriction enzymes for identifying places at which different human genomes differ, there has been a welcome growth of interest in the field. International networks of psychiatrists are alert to the importance of identifying patients whose families may provide useful information about the inheritance of psychiatric disease. The discovery two years ago of a genetic basis for manic depression among a large Amish family (*Nature* 325, 783; 1987) was an important landmark. It goes without saying that psychiatry, for too long one of the Cinderella specialities of medicine, will profit enormously from the availability of objective tools for study. But it is important that the objectives of these developments should not be generally misunderstood. The search for the genetic correlates of psychiatric illness is not predicated on the view that all psychiatric illness is simply and genetically determined. Rather, it is that genetic studies, among the families in which it is known that a susceptibility is inherited, may provide a route to the understanding of mechanisms and even to the definition of the non-genetic factors that may influence the development of disease.

One of the practical benefits now within grasp is typified by Lander's emphasis of the link emerging from the London study between schizophrenia and other psychotic conditions. This is especially important. The clinical definition of schizophrenia has long been a perplexing issue (and even now there are disconcerting differences between the definitions in common use in the United States and Europe, for example). The differentiation between schizophrenia and other psychotic conditions is often fuzzy. What the Icelandic families appear to show is that the link



between the inheritance of a defective gene and its carriers' predisposition to schizophrenia may be part of a more complicated story. If it should eventually be shown that those inheriting the defective gene inherit a predisposition to several psychiatric conditions, among which schizophrenia is merely one, the result will be of great benefit in the understanding of psychiatric illness in general.

Objections

Investigations of this kind would also help to resolve one of the abiding objections to previous theories that schizophrenia in general is genetic in origin. Briefly, the frequency of the disease is so great and its consequences so handicapping that, if a substantial proportion of overt schizophrenia were genetic, the underlying trait would long since have been eliminated from the human population by natural selection. (In present social circumstances, the fertility of people with schizophrenia is below average, if only because the disease inhibits those who suffer from it from forming close relationships.) Lander's advocacy of a search for what he calls the biological correlates of schizophrenia might, among other things, yield some understanding of where, in the behaviour of those carrying the presumptive abnormal genes but not affected by overt schizophrenia, are the offsetting increases of darwinian fitness that have allowed genes for schizophrenia to persist.

One contentious side-issue that may be settled outright by the research now reported is that best described as the relativistic definition of madness. On this view, most often linked with the psychiatrists R. D. Laing and, separately, Dr Thomas Szasz, there is no such thing as madness, but merely a range of differing perceptions of reality seized upon, by the majority, as an opportunity to label the minority as mad. The view is nicely embodied in the film *One Flew over the Cuckoo's Nest*. This view has for years provided too many opportunities for confusion in the treatment of patients with schizophrenia, not to mention opportunities for patients to decline whatever treatments have been on offer. Even the demonstration that there is a handful of patients (in Iceland) differing in measurable ways from the other members of their families should help to banish this source of confusion.

Meanwhile, it is important that the demonstration (all that has been done so far) that the schizophrenia in some Icelandic families is genetically determined should not deter those engaged in the care and treatment of schizophrenia from their often-daunting work. Some will naturally be tempted to say, "But if it is genetic, surely there is nothing that can be done?" That question and the fatalism it implies are natural enough, but are mistaken. The logical error is the assumption that the only worthwhile treatments of disease are those that are tantamount to cure. But who says that the use of insulin for the treatment of diabetes is not worthwhile?

The new situation with schizophrenia is like that in the long-standing and bitter argument about the origins of intelligence, as measured by IQ. The question of whether IQ is inherited, and if so to what degree, remains unresolved, but if it were demonstrated tomorrow that IQ is predominantly genetic, would that in any important way affect the proper education of the young? Whatever the degree to which schizophrenia is genetically determined, treatment for the foreseeable future will remain a matter of blending the judicious use of drugs with other treatments that enable patients the more successfully to live with their disabilities in the real world. The most likely immediate benefit of the new developments is that there may be more effective drugs sooner than would otherwise have been the case.

But what is wrong with the drugs already available? For have not the phenothiazines made it possible for millions of people who would previously have been locked up in psychiatric institutions to be dealt with differently, in a more humane fashion? It goes without saying that the nineteenth century prescription for

the treatment of schizophrenia is now entirely inappropriate. Locking people away in institutions is a recipe for letting the passage of time justify the diagnosis that they are mad. Much of the spur for the general reform of public policy on psychiatric illness in recent decades was the general recognition of the destructiveness of institutionalization. It was lucky, in the 1960s, that there were drugs that allowed of a more enlightened policy available at the right time. But that does not imply that the more enlightened policy, as applied, is everything that could be asked for.

On the streets

Without belittling the achievements of the past quarter of a century, it must be confessed that the now-standard treatment of schizophrenia is anything but satisfactory even in the most advanced communities. The assumptions are that when the disabling symptoms of the disease have been controlled by drugs, patients can be 'returned to the community' (one euphemism) for 'treatment in the community' (another). All too often, this entails that patients who would previously have spent years or whole lifetimes in psychiatric hospitals are decanted onto the streets to fend for themselves. Some, with support from families and friends, can do so. (One haunting feature of schizophrenia is that those who suffer from it are paranoically alienated from their families, while their capacity for even ordinary friendship is diminished.) Others cannot, or cannot manage all the time. They are the patients who are now the familiar informal residents of all major cities, sleeping rough in the neighbourhood of railway stations and other such places where aimlessness may be unremarked. The population of the psychiatrically derelict may be smaller than the former population of the psychiatric hospitals, but is none the less an offence against civility of that account.

For the best of reasons (enlightenment), governments in advanced communities have embarked cheerfully on the shutting down of psychiatric institutions — and their treasuries, just as cheerfully, have calculated the fiscal economies of enlightenment. Rarely have policies for the treatment 'in the community' of schizophrenia patients been matched by a sufficient commitment of resources to community care.

Mere niggardliness is only part of the explanation. Governments everywhere save money where and when they can. It is also relevant that the most enlightened communities are still shamefully, yet studiously, reticent in their regard for schizophrenia and for psychiatric illness in general. On the face of things, the evidence now brought to light from Iceland may increase the risk of reticence. Part of the trouble has always been the suspicion that psychiatric disease may run in families (whence people's eagerness to lock away affected relatives). Will it not be worse now that there is some evidence, however scant, for genetic causation?

Understanding

The other side of that coin is enlightenment of a different kind. One of the side-benefits of the breathtaking advance of molecular genetics in the past few years has been the beginning of a general understanding that individuals are not personally responsible for the genes they and their relatives happen to be carrying. Even the concept that an apparently harmful gene (such as that which causes sickle-cell anaemia in homozygotes) may have beneficial consequences (such as protection against malaria in carriers of the sickling gene) is becoming generally familiar. Is it now unreasonable to hope that people who have fled from the notion that psychiatric illness may be familial may be more willing to acknowledge that the truth may be otherwise when they are shown that there are also familial benefits? That must be everybody's hope, for otherwise the social burden of psychiatric illness will remain partially obscured and, therefore, unrelenting. □

Networked computers hit by intelligent 'virus'

- Top secret computers safe from attack
- Cornell University tracks virus' creator

Washington

THE vulnerability of networked computers to attack by computer 'viruses' made world headlines last week as thousands, possibly tens of thousands, of computers using the UNIX operating system were infiltrated via electronic mail. Although the virus did not destroy any data or compromise programs, the cost of removing the flaw that allowed virus infection will be substantial.

When the virus was first detected at computer installations around the country on Wednesday night last week, worried system operators took drastic measures to isolate their computers from the Internet network that was transmitting the virus. Such measures, although appropriate at the time, turned out to be unnecessary. Stephen Wolff, director of the division of networking, communications research and infrastructure at the National Science Foundation (NSF), says the virus was totally innocuous, merely making a copy of itself and moving on to the next system. But some systems slowed down substantially or crashed as the virus searched files in an attempt to carry out its mission.

The virus only affected machines running the Unix operating system, and only the version known as Berkeley Unix. Once released last Wednesday, the virus traveled over the ARPANET, Milnet and NSF Net computer networks, establishing itself at host computers. The program took advantage of a debugging option included with some distributions of the electronic mail program "sendmail" that allowed binary code to be introduced into the host machine. Using loopholes in the Unix security system, the virus obtained access to machines and accounts that would normally be inaccessible without the proper password. Wolff described the virus program as "very clever". It encrypted its instructions, decrypting them line by line as they were executed.

Once its route of attack was determined, NSF prepared a simple patch to the mail program and distributed it to network users. With the patch in place, Wolff says the systems are immune from that particular mode of attack.

The virus was apparently created by Robert T. Morris, a graduate student in computer science at Cornell University in Ithaca, New York. A Cornell spokesman says the university has found a file in Morris' computer account with some of the same passwords that were found in

versions of the virus recovered from remote machines. The campus is now going through backup tapes to try to follow the course of the virus' development.

Morris, who has only been a graduate student at Cornell since the start of this year, left campus last week to go to the home of his father, Robert T. Morris, Sr., who, ironically, is chief scientist at the National Computer Security Center, a division of the National Security Agency.

Jeffrey Schiller, Hardware Manager at Massachusetts Institute of Technology (MIT), said unlike other viruses that have done damage in the past, this virus was "completely automated" in the sense that it decoded user passwords, and replicated itself on new machines without any user interface.

Most systems affected by the virus were back on the Internet by Friday last week. Officials at the Los Alamos and Lawrence Livermore National Laboratories said both installations were affected, but that the top secret computers containing highly classified information are not accessible from the networks over which the virus travelled.

ARPANET and Milnet were both originally established with funding from the Department of Defense, and both are heavily used by various Pentagon agencies. A Pentagon spokesman said that many defence installations were infected, but all were in the process of implementing fixes that would eliminate the flaw that allowed the viral attack.

A spokesman for the National Aeronautics and Space Administration (NASA) said that several NASA facilities including the Jet Propulsion Laboratory in Pasadena California, and the Ames Research Center at Moffett Field, California were affected.

Had the virus program been written slightly differently it might have gone undetected for a long time. According to James D. Bruce, MIT professor of electrical engineering and vice-president for Information Systems, a programming error caused the program to replicate rapidly, consuming large amounts of computer time, causing users to experience sluggish performance. Had the code caused the program to suspend operation for a time after arriving at a new host, or operate in the background, its presence would scarcely have been felt.

Joseph Palca & Seth Shulman

Sakharov to stand for Supreme Soviet?

London

ACADEMICIAN Andrei Sakharov — at present visiting the United States — may stand for election to the Supreme Soviet (parliament) of the USSR in the multi-candidate elections promised for next March. Sakharov's candidacy was proposed during the recent conference of *Memorial*, the "informal" society established recently in Moscow which plans to erect monuments and a "memorial complex and documentation centre" honouring the victims of Stalin's purges. Soviet political theorists do not envisage a multi-party system, but support the idea of candidates from special interest groups, such as *Memorial*, so long as their aims do not conflict with the Soviet system.

Addressing the *Memorial* conference in Moscow, Sakharov, who is honorary chairman of the society, said that not only the victims of Stalin, but also those of Khrushchev and Brezhnev's repressions must be exonerated and honoured. He urged the release of all persons detained in psychiatric hospitals on account of political activities.

Recent statements by Soviet officials claim that all such persons have already been released or are having their cases reviewed. Psychiatric prison hospitals have now been closed and those unfortunates still detained in "ordinary" psychiatric hospitals are, it is maintained, genuinely disturbed.

Vera Rich
■ US-Soviet talks on psychiatric abuse, page 98.

Smokeless cigarettes in trouble

Washington

THE American Medical Association is asking state medical authorities to halt the test marketing of 'Premier', the world's first smokeless cigarette. Premier 'cigarettes' consist of an aluminium tube containing nicotine-impregnated 'flavour pellets' heated by a slow-burning carbon tip. Although the cigarettes are free from carcinogens, the American Medical Association contends that they are potentially dangerous as delivery systems for the addictive drug nicotine.

Premier is now on test sale in Tucson, St Louis and Phoenix at a price about 25 per cent higher than that of conventional cigarettes. The federal Food and Drug Administration is also considering pleas for a review of Premier's safety. Premier has been developed by the R. J. Reynold's tobacco company, manufacturer of Winston cigarettes, partly in response to a decline in conventional cigarette sales.

Alun Anderson

Long-awaited French anti-AIDS campaign is launched

Paris

THE French Minister of Health, Claude Evin, has finally disclosed government plans to step up national efforts to combat AIDS. The announcement follows a seven-month period without public health campaigns or response to complaints of financial asphyxiation from researchers and hospital staff. But the news is worth the wait: a tripling of government grants for AIDS research, quadrupling of funds for public health education, extra money for hospital care of AIDS sufferers, payment of damages to haemophiliacs infected by contaminated blood products and the creation of two non-governmental coordinating committees.

Reactions to the new AIDS programme have been favourable, with a general feeling that the government has finally appreciated the scale of the epidemic in mainland France. If the disease continues to spread at present rates (doubling every 11 months), there could be over 20,000 declared AIDS cases by the end of 1989.

The budget for health education will be three times last year's, at FF100 million (\$16.13 million), of which FF50 million will be spent on national information campaigns in the media. A television campaign to promote use of condoms is scheduled for the end of November. The condom is still an unpopular and relatively expensive form of contraception in Catholic France and sales have been impeded by a jealously guarded pharmacy monopoly on sales of surgical and drug products. In addition to media campaigns, a further 4,000 health education specialists will be trained during 1989 to conduct campaigns centred on secondary school pupils.

With the priority of prevention in mind, Evin is aware that AIDS is spreading most rapidly among intravenous drug-users, particularly in the Paris area and in densely populated cities in the south of France. Evin will extend the decision taken by the last Minister of Health, Michèle Barzach, to make disposable syringes available over the counter in pharmacies. Systematic national screening for human immunodeficiency virus (HIV) is still being ruled out. But Evin wants blood tests to be routinely proposed to pregnant women believed to be at risk and to all new prison detainees. Condoms will be available in prisons, on request, from the prison doctor.

The cost to the health service of treating AIDS sufferers could cripple state health care, if the projected figure of one state hospital bed in six occupied by an AIDS patient within five years is realized. Evin has released an extra FF430 million for

hospital care and has created an extra 200 new jobs, 30 of which will be reserved for medical practitioners. This is unlikely to be adequate, so Evin has set up a special working group to study the future impact of the epidemic on the health service.

The AIDS research budget will rise to FF150 million from FF50 million. But the figures are misleading. In May, Luc Montagnier of the Pasteur Institute complained that government grants for AIDS research had dropped from FF150 million to FF20 million. Although this sum was later apparently boosted to FF70 million, both the health and research ministers said that it was pointless to give more money because it was not being spent. About FF50 million of the 1987 AIDS research budget was, indeed, left in the bank at the end of the year and was carried over to 1988. But one leading researcher says that this was because the research ministry took a year to pay its bills, not because the money had not been spent.

Evin is proving a believer in working groups and committees. To oversee AIDS research spending, he will set up a non-governmental science committee "to stimulate, coordinate and evaluate" research in all sectors. He hopes its membership and scope will be Europe-wide, if other countries agree. A national AIDS committee will also be established, comprising 15 as-yet unnamed specialists, to "discuss, to propose and to recommend". The ministry of health will also have a special agency to oversee public health campaigns.

Peter Coles

ESA science projects due to be chosen

Paris

FIVE projects competing for a place in the European Space Agency's (ESA) Horizon 2000 long-term space science plan were presented in Bruges, Belgium last week. A final choice of one project will be made at ESA's Science Programme Committee meeting on 24 and 25 November. The five projects are:

CASSINI — a Saturn orbiter and Titan probe;

GRASP — gamma-ray astronomy with spectroscopy and positioning;

LYMAN — High resolution spectroscopy in the 900–1,200 Å band;

QUASAT — a space VLBI satellite;

VESTA — missions to asteroids and comets in the solar system.

Following design and development starting next year, the selected project should be ready for launch in 1996.

Peter Coles

US–Soviet talks on psychiatry

Washington

THE latest step in a complex dance between the United States and the Soviet Union over alleged abuses in Soviet psychiatry is scheduled to take place this week; a small but official delegation from the United States is visiting the Soviet Union to work out logistics for a visit by a formal investigating team early next year. Although the visit makes US officials optimistic that the debate over abuses can be resolved, they say there is still a long way to go before psychiatry can be added to the disciplines in which the two countries can collaborate.

Ill feeling between the two countries reached a peak in 1983 when the All Union Society of Psychiatrists and Neuro-pathologists of the Soviet Union resigned from the World Psychiatric Association (WPA) because of efforts by the Ameri-



can Psychiatric Association (APA) to have the society expelled for psychiatric abuses. In the past few years, Soviet health officials have sought to remove US objections to Soviet psychiatric practices, and have tried to invite US health officials to inspect Soviet facilities. But the United States has asked that an APA representative be included in any delegation.

There appears to be a shift in Soviet attitudes. The Soviet Union has reapplied for admission to the WPA, and has accepted an APA representative as a member of the visiting team now being assembled. The visit will be held under the auspices of the US State Department, with representation from the National Institute of Mental Health. The State Department believes that the flexibility the Soviet Union is now showing is intended to create a good impression with WPA. Loren Roth, a forensic psychiatrist from the University of Pittsburgh, will lead the delegation.

By a coincidence of timing, at the same time as the logistical team for the psychiatric visit is in the Soviet Union, a delegation from the US Department of Health and Human Services is in Moscow to review progress and plan new activities under their joint health agreement, but US officials stress that psychiatry will not be discussed.

Joseph Palca

Government action needed to mitigate 'greenhouse effect'

London

THIS week is a busy one for researchers, environmentalists and policy-makers concerned with the predicted increase in global temperatures as a result of the 'greenhouse effect'. In Geneva, the inter-governmental panel on climate change set up by the World Meteorological Organisation and the United Nations Environment Programme (UNEP) meets for the first time; its remit is to evaluate current scientific knowledge on the subject, determine to what extent climate change can be slowed down and formulate policies to adapt national economies as necessary. And a conference in Hamburg — billed as the follow-up to the Toronto conference on the changing atmosphere held in June (see *Nature* 333, 691; 1988) — is planning to formulate policies to tackle the problems of the 'greenhouse effect', acid rain and the destruction of the ozone layer. This action plan has already been dubbed the 'Hamburg Manifesto'.

The scientific evidence presented at both events will include a comprehensive global study of the effects of climatic variations on agriculture. This study, made available last week, was carried out for the International Institute for Applied Systems Analysis and UNEP, under the direction of Martin Parry of the University of Birmingham. The work was carried out over a period of four years by a team of 76 researchers from 50 institutions in 17 countries.

It covers not only the effects of short- and long-term climatic variations on agricultural productivity, but also the effect on regional and national economies. And it considers possible adjustments to mitigate these effects. Parry urges that such adjustments must be made in advance of changing conditions rather than as a response to them.

The scenario for climate change in the long term is based on a possible increase in global mean temperature of 1.5–5.5% between the years 2050 and 2100; the temperatures are derived from the Goddard Institute for Space Studies general circulation model.

Five areas in the cool temperate and cold regions of the world were studied: Saskatchewan (Canada), Iceland, Finland, the northern European Soviet Union and Japan. The results of the Saskatchewan study indicate that a temperature increase could have a disastrous effect on the prairie wheatbelt of the United States and Canada. This area could return to the 'dustbowl' conditions of the 1930s, with severe droughts occurring more frequently. A dramatic setback in farm production could lead to the loss of about 4,000 jobs

and a loss in income of about Can\$500 million annually.

The outlook for the Soviet Union in the areas studied is less grim. Climate warming could improve crop yields significantly if crops more suited to the warmer climate were grown, although, in the long term, increased precipitation in the western region, around Leningrad, could lead to declining crop yields, because of soil degradation and waterlogging of fields.

The problem for Japan will be different: a temperature rise will favour increased production of rice leading to an acute surplus. The price of rice, grown on four million farms, is determined by the government and in recent years has been three to four times higher than world prices. A significant increase in production would require a change in traditional government policies towards minimized intervention and a liberalized rice market, says the report.

Agricultural policies in northern Europe might also need some reconsideration: the studies of Finland and Iceland indicate that increased crop yields in northern Europe will exacerbate current problems with expensive agricultural policies that subsidize farm production and exports of surplus products.

At lower latitudes, where most of the semi-arid areas studied are located, a temperature increase resulting from the greenhouse effect is expected to be significantly smaller than at middle and high latitudes. And there are still great uncertainties in estimates of changes in precipitation, which is of great importance in these regions. So researchers focused on short-term climatic variations, especially droughts.

The areas studied were central and eastern Kenya, northeast Brazil, the central sierra of Ecuador, dry tropical India, the Stavropol territory and Aratov regions of the Soviet Union and the Australian wheatbelt. Among the outcomes of the studies was the prediction that the El Niño phenomenon which was linked in 1982–83 with disastrous droughts in semi-arid regions of Brazil, Australia, India and parts of Africa may become more frequent. And severe shocks to agriculture from floods, persistent droughts, soil erosion, forest fires and crop pests may become more intense. Recommendations made include better use of land, resources and of new technology in order to increase agricultural production and the development of a consistent set of draught policies.

This work is being followed up by similar studies for Europe and South-East Asia.

Christine McGourty

More money for science in Ireland

Dublin

IRELAND plans to spend three times as much on science and technology next year as this, despite continuing reductions of general expenditure. Estimates released on 25 October show an increase from IR£3.1 million to IR£8.2 million for science and technology, while general spending is to fall by IR£224 million.

The Minister for Science and Technology, Dr Sean McCarthy, said that the increase reflects the government's commitment to "investing in the people who can design new products . . . research new opportunities and apply science and technology to new areas of development".

The largest allocation of funds (IR£2.25 million) is to the national biotechnology programme. Two further biotechnology centres will be set up at Trinity College, Dublin, and University College, Dublin. Last year, the programme, which began in 1987, received IR£1.5 million and set up three centres of excellence, in diagnostics, cell culture and food technology.

Despite the increased budget, grants to colleges will fall by 1–3 per cent, and research grants and fellowships to the universities will fall by over 10 per cent, leaving scientists to compete even more intensely for the limited pool of contract funding available.

Mary Mulvihill

Steps towards a new science policy

Barcelona

ON his return from the Seoul Olympics, where he was representing the Spanish government, the Minister for Education and Science unexpectedly reshuffled some of the main science policy offices. Dr Emilio Muñoz, secretary general of the National Plan for Research, has been appointed president of the science research council (CSIC), he in turn was replaced by Dr Luis Oro, formerly director general of science policy, a position now taken by Dr Pedro Ripoll, a geneticist from the Centro de Biología Molecular in Madrid.

The extent of the changes came as a surprise. Most of those previously involved in the direction of CSIC will leave their posts and will be replaced by former colleagues of Muñoz in the national plan. Thus two of the principal institutions in Spanish research will see major changes in their senior positions. These changes will deeply affect the conduct of research policy in the coming years.

Muñoz, a molecular biologist from the CSIC, says he will try to improve CSIC's relations with universities and with private industry, two key issues just now.

Pedro Puigdoménech

Japanese companies bring halt to imports of uranium

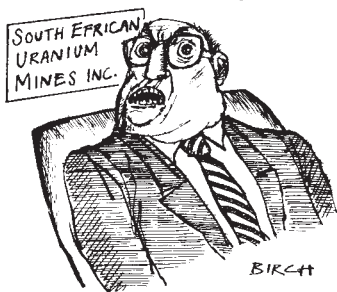
Tokyo

IN response to international and government pressure, several of Japan's giant electric power companies decided last week to halt imports of uranium from South Africa and Namibia. The two countries are major suppliers of fuel for Japan's huge nuclear power industry. But Tokyo Electric Power Company, the world's largest electric utility, says it will continue imports from Rio Tinto Zinc (RTZ), a British mining company which derives much of its uranium from Namibia.

At a time when many Western countries are clamping down on trade with South Africa in protest against apartheid, Japan last year found itself in the embarrassing position of being South Africa's number one trading partner with a two-way flow in trade of over \$4,000 million. Trade in dollar terms is expected to be even higher this year and Japan has come under pressure from its allies to impose stricter sanctions.

Japan's Ministry of International Trade and Industry and the Ministry of Foreign Affairs have been trying for two years to persuade electric power companies to end South African uranium imports. The

Sech ingratitute, after we made them honorary Whites!



United States banned imports of uranium from South Africa in 1986. This year, the United Nations issued a decree prohibiting imports from Namibia, which is governed by South Africa in defiance of a United Nations resolution.

The big electric power companies in Japan have argued that they cannot cancel long-term contracts. But they have now decided they will not renew them. On 1 November, Tohoku, Kansai, Kyushu, Chubu and Chugoku electric power companies jointly announced that contracts with South Africa and RTZ will be allowed to lapse between 1989 and 1995.

As at March 1987, about 11 per cent of Japan's uranium imports, or 22,000 short tons, was derived from South Africa. But virtually none of the ore was imported directly. Japan has only very limited faci-

ties for enrichment and most of the uranium was purchased as uranium hexafluoride and shipped to France and the United States for enrichment — despite an import ban on South African uranium ore, the United States continues to enrich partially processed uranium of South African origin.

Japan also obtains considerable amounts of uranium from RTZ, whose uranium mine in Namibia is one of the largest open-cast operations in the world, according to John Skinner, press officer for RTZ in London. For example, by 1989,

Chubu Electric Power Company will have imported nearly 4,000 short tons from RTZ under a 12-year contract. But RTZ also operates a large uranium mine in Canada and precise figures for Namibian imports are not available.

Despite uncertainty over the origin of RTZ's uranium, Tokyo Electric Power Company, which operates 11 out of Japan's 36 nuclear power plants, says it will continue imports from RTZ. A company spokesman says RTZ has provided a written guarantee that none of the uranium supplied will be of Namibian origin. But Skinner says he can "neither confirm nor deny" the existence of such an agreement. RTZ, as a matter of policy, does not discuss its commercial contracts, he says.

David Swinbanks

Protests in Japan about trade in tropical forest timber

Tokyo

"STOP Japan's chainsaw massacre in Sarawak". This sign greeted passers-by in downtown Tokyo as environmental groups launched a one-day campaign on 31 October to protest against Japan's involvement in the tropical timber trade.

Japan imports nearly half of the world's tropical timber, making it by far the biggest consumer. But most Japanese are totally unaware of this fact according to Yoichi Kuroda of the Japan Tropical Forest Action Network (JATAN). The bulk of the timber comes from Sarawak, Malaysia's eastern state, where logging has destroyed more than 30 per cent of the total forest area.

In Tokyo environmentalists submitted a petition and letter calling for a halt to tropical timber imports to Prime Minister Noboru Takeshita, the Ministry of Agriculture, Forestry and Fisheries, the Ministry of Foreign Affairs and companies involved in the import of tropical timber, including C. Itoh and Nissho Iwai.

Coordinated protests took place in more than twenty countries. Demonstrations were staged outside Japanese embassies and Japanese trading companies. The protests were intended to coincide with the day when 42 Kayan tribespeople from Sarawak were to go on trial in a Malaysian court for blockading a timber road to protest logging activities. The trial has now been postponed until April.

Outside Malaysia, Japanese companies are involved in logging operations throughout South-East Asia. Marubeni Corporation is soon to begin imports of wood chips from a logging operation in western (Indonesian) Papua New Guinea that threatens one of the largest primary mangrove swamps in east Asia.

The mangrove in Bintuni Bay has been provisionally designated as a nature

reserve by the Indonesian government. But surveys of the reserve have yet to be carried out, and in 1983 a local company, P.T. Bintuni Murai Wood Industries, was given a logging concession of 137,000 hectares, about a third of which overlaps with the reserve.

Indonesian environmental regulations require preservation of a coastal strip of mangrove and isolated 'mother trees' in logged areas to give the mangrove a chance to regenerate. Kuroda says he recently asked the director of Marubeni's wood chip division if the Indonesian regulations would be followed: the Marubeni official said that the logging plan is the responsibility of the local company. Marubeni just imports the chips.

David Swinbanks

Dial-a-scientist links three continents

Sydney

AUSTRALIANS should soon be able make use of a new dial-a-scientist service. Aimed primarily at journalists, parliamentarians and teachers, the service will provide access to experts in all realms of science and help to arrange laboratory visits for schoolchildren. The service is intended to be a catalyst towards a global network and will begin by making links with the Scientist's Institute for Public Information in New York, the Ciba Foundation Media Resource Survey in London and the Science Information Sources Organization in Toronto.

The Australian Academy of Science has raised A\$50,000 for the service. An expected government grant for A\$313,000 should allow a start in 1989.

Charles Morgan

Japan barely joins SDI goldrush

Tokyo

JAPANESE companies have just won their first — and perhaps last — contracts from the US Strategic Defense Initiative (SDI).

Japan was slow to get involved in SDI and the programme may fade away before Japan has a chance to enter the SDI league table (see below). The two new contracts were awarded to Japanese consortia by the US Department of Defense for the design of a defense system to protect Japan and the west Pacific from Soviet missile attack. The one-year contracts, worth \$3 million each, will be shared by 18 US and Japanese companies. Mitsubishi Heavy Industries, a major Japanese defence contractor, leads one group of thirteen companies and LTV Corporation, a US steel maker, heads the other.

The Japanese electronics giants NEC, Fujitsu, Hitachi and Mitsubishi Electric are among the participants.

Japan trails far behind Israel and Western European countries which have

SDI contract dollars

	\$ million
Israel	161.93
West Germany	63.21
United Kingdom	44.19
The Netherlands	12.07
Italy	9.41
France	6.25
Canada	1.07
Belgium	0.09

The value of contracts let under the auspices of the US Strategic Defense Initiative Office (SDIO). The total for the Netherlands includes \$7 million from the government of the Netherlands, and the Israeli government contributed \$31.6 million to its SDI contracts. There are 122 contracts with foreign countries for participation in SDI. Data provided by the SDIO, complete to end October 1988. J. P.

won large SDI contracts. Negotiations over Japan's participation, which began nearly four years ago, proceeded slowly, partly because Japanese companies have no experience in taking out contracts with the Pentagon and partly because special measures had to be introduced in Japan to protect "military secrets". In April, Japan and the United States agreed to a system for filing classified patents in Japan. Before that all patents were made public.

Japan may now never win substantial SDI contracts, given the chances the programme will be scaled down in a new US administration. But SDI has opened the way for increased collaboration between the US and Japan in the development of military technology. David Swinbanks

Thalidomide scientist "guilty of fraud" says committee

Sydney

WILLIAM McBride, the Australian medical researcher credited with discovering that the drug thalidomide could cause birth defects, has been found guilty of scientific fraud in his research into another morning-sickness drug. McBride has resigned from Foundation 41, the institute he founded and directed.

Allegations that McBride had fabricated and altered data published in the *Australian Journal of Biological Sciences* were first made in December 1987 by Norman Swan, the Australian Broadcasting Corporation's health correspondent. McBride's paper stated that orally administered hyoscine, an anticholinergic drug, causes birth defects in rabbits. Swan claimed the real results had been inconclusive, with only one of six rabbits giving birth to a deformed litter. He said McBride had changed the results to show that more deformed rabbits were born and published the paper without the knowledge of his junior researchers.

An early draft of the paper appeared as evidence in a US lawsuit in 1981 in which it was alleged that the drug Bendectin (also known as Debendox) contained an anticholinergic agent and was thus potentially dangerous. The drug was withdrawn from the market in 1983.

Public pressure resulting from Swan's allegations forced Foundation 41 to begin an inquiry earlier this year. As the foundation is an independent organization, it turned to an outside committee chaired by Sir Harry Gibbs, a former Chief Justice,

Roger Short, professor of anatomy at Monash University, and Robert Porter, director of the John Curtin School of Medicine.

In a statement released by Foundation 41 last week, the committee said that McBride had "deliberately falsified" the disputed article. "The experiment mentioned in the paper was not conducted in accordance with proper scientific method and was not honestly reported . . . we are forced to conclude that Dr McBride did publish statements which he either knew were untrue or which he did not genuinely believe to be true, and in that respect was guilty of scientific fraud".

McBride disputes the verdict. In an interview broadcast on Sydney's Channel 7 news, he said that "by the way the committee concluded its affairs I was denied natural justice . . . the terms of reference [of the inquiry] were very narrow. I deny the findings of the committee. I had nothing to gain by falsifying data. I am not guilty of scientific fraud."

McBride may now be investigated under the Medical Practitioners' Act of 1987. Although the act deals with medical rather than scientific improprieties, Marilyn Walten of the State Health Department's complaints unit says that McBride could face deregistration as a medical practitioner on the grounds of not being "a fit and proper person to practise medicine". A recommendation on further action will be presented to the medical tribunal within the next three weeks.

Tania Ewing

Ethical issues in vogue at MIT

Boston

THE growing interest in issues of ethics in science and technology was underlined by a standing-room-only crowd of 850 students, faculty and staff from the Massachusetts Institute of Technology (MIT) at a university-wide colloquium last month entitled "How to be Good". Following the formal presentations, dozens of workshops continued to discuss the issues raised well into the night.

Charles Whetsel, an undergraduate senior at MIT, and one of the organizers of the event, says that the ethics colloquium was intended to "get the ball rolling" on a subject that is "very much in the forefront of people's minds". Although no official proposals have yet been offered, many faculty and administrators are debating the issue of how better to integrate ethical issues into the school's curriculum.

Sheila A. Widnall, professor of aeronautics and astronautics and past president

of the American Association for the Advancement of Science, argued that courses on ethics and increased insertion of ethical issues into regular course curricula are needed to raise student awareness of ethical issues. Faculty members at MIT, she said, have "a responsibility to set an academic climate where one is not pressured into dishonesty to maintain one's academic standing". Philip Morrison, emeritus professor of physics, spoke about two key decisions he had made in his career that required him to reconsider his own values. He contrasted his decision to participate in the creation of the atomic bomb in the Second World War with his decision to not to work for the Rand Corporation. The one, he said, was an effort to thwart "the extension of fascism throughout the whole world", while the other would have meant studying "waging of intercontinental warfare by any and all means".

Seth Shulman

India and United States quarrel over intellectual property rights

New Delhi & Washington

NEITHER India nor the United States seems likely to compromise in a worsening quarrel over the protection of intellectual property rights. Many joint research projects launched under the recently renewed joint Science and Technology Initiative are now at risk.

US negotiators claim that the Indian patent system does not protect inventions that may arise in joint research. But India, which is not (and does not wish to be) a party to the Paris Convention, maintains that inventions can be protected adequately under the prevailing laws in each country.

In April 1987, President Reagan ordered US agencies to sign science agreements only with countries that protect US intellectual property rights. "Since then we had been under tremendous pressure from the United States to change our patent law but we did not buckle", a spokesman of the Indian Department of Science and Technology (DST) said.

Because of the quarrel, William Graham, Reagan's science adviser, twice postponed a visit to India to renew the Science and Technology Initiative. The agreement was eventually signed on 5 October, but only after a personal letter was sent from Prime Minister Rajiv Gandhi to President Reagan, and a decision made to resolve remaining differences through bilateral discussions. A US negotiating team will visit India later this month.

Indian patent protection concentrates on the process of production, rather than the product, enabling Indian companies to manufacture any item provided they are able to create a new production process. Product patents are granted only in selected fields for periods of 7 to 14 years. Atomic energy and living organisms cannot be patented at all.

The United States is asking for changes that will grant 20-year product patents in all fields to prevent what it calls "thievery of intellectual property". But the Indian government claims that its system has helped the country produce cheap drugs and agrochemicals and fears that changes would hamper industrialization, boost drug prices and shut off foreign markets.

Graham warns that the Indian patent regime is an impediment to transfer of high technologies and will harm the Science and Technology Initiative. If joint research "is to move from basic science to the marketplace", India's system must be aligned with that of the United States, he says. The controversy also threatens progress on the Vaccine Action Programme under which Indian and US scientists will

jointly develop and test genetically engineered vaccines (the vaccination programme faces other hazards, see *Nature* 335, 584).

In India, opposition to change is strong. Gandhi's science advisory council has cautioned that "nothing should be done to undermine the supremacy of the Indian patents act". And industry has formed a national working group to counter US pressure.

In Washington, trade officials are eager to force India to compromise. In late October they were successful in imposing import tariffs on Brazil as a punishment for that country's unwillingness to grant patent protection to US pharmaceutical and chemical products. But the US State Department opposes such tough action with India, given its strategic importance and the links it has forged with the Soviet Union. A more likely outcome is that the United States will threaten to back out of some joint high-technology projects.

K.S. Jayaraman & Alun Anderson

IRL Press becomes an OUP acquisition

London

A FIGHT for a highly successful publisher of textbooks and journals in the biomedical sciences has ended with the University of Oxford emerging as the winner. The university's publishing company, Oxford University Press (OUP), has taken over IRL Press, well known as the publisher of the journals *Nucleic Acids Research* and the *EMBO Journal*. The price paid has not been disclosed.

IRL plans to build on its success, using the wider marketing network of OUP to increase the circulation of its journals. The company plans to move out of its narrow subject range and to publish more textbooks; it will retain its name.

OUP, which is more than 500 years old, plans to expand into journals and textbooks from its main role as a publisher of scholarly works such as theses, and to specialize in certain areas. The main aim of OUP is charitable and profits from IRL will help to support less profitable sectors. OUP has no overall profits; it exists entirely on cash flow and reserves, and is exempt from corporate tax.

The take-over has been "relatively painless", says John Bradley of IRL. The company was in a strong position to ensure favourable terms for its staff and those currently working on the journals of OUP will move to the premises of IRL, near Oxford.

Christine McGourty

Student Chinese overstaying in United States

Washington

STUDENTS and researchers from the People's Republic of China are flocking to US universities in unparalleled numbers but many are putting off their return home. This pattern is causing considerable concern in China that the best and brightest students may be lost to the United States, and not a little anxiety in the United States that engineering schools are too dependent on Chinese researchers.

A report* from the National Academy of Sciences released last week estimates that there are 29,000 students and researchers from the People's Republic in US universities. The majority are in engineering and science faculties; statistics from the National Academy of Sciences show that more engineering doctorates are granted to students from China than to the students of any other two foreign nations combined. With close to 50 per cent of engineering doctorates now going to non-US citizens, that gives the Chinese a formidable presence.

Many Chinese researchers and students are now adopting a "wait-and-see attitude", according to the report, attempting to postpone their return while watching political developments in China. Recent student demonstrations in China and the dismissal of outspoken academics, such as Fang Lizhi of the University of Science and Technology in Hefei, "visibly alarmed" the student community abroad.

But political worries are not the whole story. Between 1978 and late 1986 almost all of the 19,500 officially sponsored students and researchers who had completed their courses went home. But Chinese students are now much more at home in the United States, and much more knowledgeable about US immigration law. The shift from Mao's slogan of "serve the people" to today's "it is all right to be rich" means, the report says, that there is a "mood of selfishness" that leaves out a sense of obligation to return home.

But the report sees nothing alarming in these trends. Although Beijing is enacting new measures to try to make sure foreign students return (and trying to increase the tiny number of students going to Europe where visa regulations are much stricter), the brain drain is likely to reverse naturally as China continues on the road to modernization. The pace of modernization may even be speeded up by foreign students who refuse to return home if proper use is not made of their skills.

Alun Anderson

* *Chinese Students in America: Policies, Issues and Numbers*. National Academy Press, Washington, DC, 1988.

NIH on the market

Washington

THE sales pitch in the hotel ballroom sounded familiar: let us show you "how to put our know-how to work for you". What made the pitch unusual was that the speaker was not an official from a private research organization, but Don Newman, number two man at the Department of Health and Human Services, and what he was selling was the 'know-how' of government researchers at the National Institutes of Health (NIH) and the Alcohol, Drug Abuse and Mental Health Administration (ADAMHA).

Newman was making his offer at the first ever NIH/ADAMHA-Industry Collaboration Forum, held last month. The forum was a direct consequence of the Federal Technology Transfer Act of 1986 which promotes cooperative arrangements between industry and federal laboratories. Over a hundred research projects were offered up on posters as potential areas for collaboration, and some 250 representatives of industry were on hand to sift through the collection, looking for projects to fit in with their research plans.

The mechanism NIH hopes will make these collaborations work is the Cooperative Research and Development Agreement. Under these agreements, companies can negotiate for an exclusive licence to any marketable products that come about as a result of a collaboration. Philip Chen, NIH associate director, says this new mechanism will make it easier for industry to enter into agreements with federal researchers, avoiding much of the red tape that hindered collaborations in the past.

Chen is encouraged by the turnout for the first forum, and expects that these forums will become an annual or possibly semi-annual event. But he agrees with some participants who felt that there are still some problems with the format. Many of the posters were too 'scientific', and did not provide any suggestion of commercial applications for the research, and some complained that there was not sufficient advance notice of what would be on display for companies to bring appropriate experts to evaluate the research.

Newman suggested in his opening address that US competitiveness would be improved if companies would take advantage of research done in federal laboratories. But attending the forum were companies with headquarters in Japan, West Germany, Italy, Canada and the United Kingdom, as well as the United States. Newman explained that as all the foreign companies present have US subsidiaries, they would provide jobs for US workers if they entered into collaborative research projects. **Joseph Palca**

More money is found for UK research councils

London

THE government added £300 million to the science budget of the research councils last week. Not all of that money will go towards building up the science base, but enough of it to satisfy some of the critics of the government's past record on science spending that a change of attitude towards science is taking place.

The money will be spread over the next three years. Next month The Advisory Board for the Research Councils (ABRC) will advise the government on how it should be distributed. The fear of the research councils will be that a significant proportion of the money may be directed to specific projects desired by the government, such as the Medical Research Council's AIDS research programme. In its advice to the government in July, the ABRC said that about £150 million would be needed over the three years to support the AIDS programme, the British Antarctic Survey, the British Geological Survey (BGS) and to pay the country's contributions to the European particle physics laboratory, CERN (see *Nature* 334, 279; 1988). But the government may choose to spend less than £150 million on these projects. This week it said it would spend only £12 million on the BGS, compared to the £42 million called for by the ABRC, and it is still looking for ways to reduce its contribution to CERN, which the ABRC says will require £27 million over the next three years.

Even assuming these projects were funded in full, the research councils will have an extra £50 million to distribute annually. But the ABRC had asked for significantly more. It said that £379 million was needed for the councils alone, and that money earmarked for specific projects should be provided separately. Of the money it wanted for the councils, about half would not be supporting research, but would be spent on restructuring the councils to make them more efficient and effective.

But Sir David Philips, chairman of the ABRC, said he was happy with the money awarded. It would provide sufficient extra money for many of the scientific initiatives proposed by the ABRC and would enable a start to be made on implementing an effective strategy for the future development of the science base, he said. The board is likely to direct a significant amount of the money to setting up interdisciplinary research centres to which it gives high priority. To set up 30 such centres over the next three years would require £105 million.

The extra £95 million which will go on the science budget for 1989-90 brings the total spending to £825 million, an increase

of 16 per cent over the figure for 1988-89. Jeremy Bray, Labour Party spokesman for science and technology points out though that if earmarked money is subtracted, the increase amounts to only 3 per cent. Denis Noble of the pressure group, Save British Science, says that this is the first time the government has planned a real increase in funds for science; it marks a change in mood within the government and marks a victory for the science lobby. Also announced last week was £14 million to be spent immediately by the research councils and the universities on equipment for basic science. The committees reviewing provision of chemistry and physics in universities had each asked for £36 million urgently.

Pressure is not off the universities to find funding from other sources. They have been awarded an extra £100 million over three years, meaning a period of level funding. The new Universities Funding Council will distribute £1,672 million in 1989-90.

Christine McGourty

Soviet criticism of Hungarian dam

Budapest/Bratislava

A LEADING Soviet economist has sharply criticized Hungary's plans to divert the river Danube and build a massive hydro-electric plant at Nagymaros. Speaking at a floating conference on board *M/V Rousse*, Academician Boris Laskorin criticised the Hungarian government's view that the project is now too expensive to stop. He argued that the long-term impact of the dam on the environment will have economic consequences that have been ignored.

M/V Rousse made its way along the Danube from Rousse in Bulgaria to Passau in West Germany carrying participants in 'Ecodanube-88', a conference on the future of the entire Danube basin. The sponsor is 'Ecoforum for Peace', a non-governmental organization set up in 1986 to unite scientists from East and West around ecological problems on an interdisciplinary basis.

The greatest concern over the environmental consequences of the Nagymaros plant and a sister plant at Gabčíkovo in Slovakia was expressed by Viktor Romanenko of the Ukrainian Academy of Sciences, who earlier this year led an expedition monitoring Danube pollution. Although he was careful not to attack the project directly, he stressed that newly-available data from Soviet rivers show that large dams on rivers in flat terrain are damaging and unlikely to meet their costs in electrical output. **Vera Rich**

Design of Thames Barrier

SIR—F. Kenneth Hare and Kenneth Mellanby (*Nature* 334, 646; 1988), adduced arguments for 'jumping the greenhouse gun' which are fortuitously linked by a recent study¹ at the Institute of Hydrology. The divergent responses to the greenhouse effect of Hare the scientist and Hare the government adviser are a current manifestation of an old problem; one which is neatly illustrated with the question of the Thames Barrier raised by Mellanby.

The same distinction existed between (a) the design criteria for the estuary defences seaward of the barrier and (b) the design standard adopted for the barrier dimensions and landward defences (the barrier is one element in an integrated scheme for protecting the entire tideway). Case (a) was scientifically based on the 1,000-year return period level in the year 2030. By contrast, case (b) was arrived at by simulating the consequences of versions of the February 1953 event, the most severe of which consisted of adding 6 ft (1.83 m) to the 1953 high water. Herein lies the source of the dichotomy; in the seaward case (a), the technology and data existed for a formal solution of the design problem. For the barrier itself and upriver, the complexity of the situation — joint fluvial influences and barrier operation — surpassed available statistical design techniques: designers therefore proceeded on the basis of a suitably severe 'scenario', the alternative of delaying construction until design technology 'caught up' rightly being unthinkable.

Mellanby feels misled about whether or not the greenhouse effect was considered in the barrier design. The study by the (then) Institute of Coastal Oceanography and Tides identified isostatic adjustment and man-made estuary geometry changes as the primary causes of a positive trend, but contemporary papers reveal that the possibility of eustatic influences was considered. Thus the short answer would appear to be that the greenhouse effect was not explicitly considered (few were so farsighted in the 1960s) but that the aggregate effect of all influences was. Design levels for the seaward reaches were projected forward to the year 2030 at annual increases of 3.8 mm at Southend increasing upriver towards 8.5 mm at Tower Pier.

The original barrier design calculations have been reviewed as part of a recent study of Thames estuary defences by this institute and Sir William Halcrow and Partners. Our updating of the high-water data and reanalysis reveals a strong downturn after 1970 paralleling the southern North Sea mean sea level trend², and little evidence for a differential trend in the upper estuary. Seasonal and other periodicities dominate secular trend in control-

ling the parameters of the distribution of high water. Upstream of the barrier, a joint probability analysis of the fluvial and tidal components (not possible at the time the barrier was designed) has highlighted the importance of the timely closure of the barrier against an uncertain surge forecast.

I am engaged in climate change impact analysis in the water resources field and my view coincides with Hare's in that as a scientist one does admit the uncertainties and unaccounted feedback mechanisms that may aggravate or ameliorate the effect. However, as an adviser, one has to be less dispassionate and attach greater weight to outcomes with the more severe consequences. In practical impact analysis in hydrology³, this means adopting some 'scenario' for change, this term generally being preferred to 'forecast' because it conveys better the 'what-if' feeling of an arbitrary although realistic and internally consistent planning tool. Climate change scenarios are based upon general circulation models, on extrapolation from contrasts between historic warm and cool periods, and by adopting analogue regions — for example, that future British river flows will resemble those current in southwest France. This is not established fact, but a working hypothesis which awaits resolution by better models (or possibly by the evolution of events).

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Leukaemia in UK

SIR—I was interested to see J.H. Fremlin's letter (*Nature* 334, 8; 1988) as I studied a cluster of leukaemia cases in Kingston, Surrey, during the 1960s in which the influence of radiation was not a factor¹. These phenomena were noted in the literature long before the advent of nuclear power stations (one of the earliest published in the United Kingdom refers to leukaemia in Cornwall in the 1940s).

Leukaemia and lymphoma in humans (as in animals) are undoubtedly associated with viruses that are probably latent and vertically or horizontally transmitted.

The emergence of leukaemia and lymphomas in transplant and immunocompetent patients has shown us that induction can be caused by immunosuppressive events, of which radiation is only one. A much more common cause might

be found in epidemics of virus infection (for example influenza) which can be immunosuppressive on a worldwide scale. It is important therefore to study these clusters, in relation not only to radiation but to the use of drugs which are cytotoxic or interfere with immune function and to the effects of local epidemics of infectious disease which may have caused immunosuppression.

There is a clear analogy with other severe illnesses (for example meningococcal meningitis) which may be precipitated in a cluster of otherwise healthy carriers by a previous virus infection².

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Philosophy fails

SIR—E. Zuckerkandl's welcome letter (*Nature* 334, 376; 1988) exemplifies the failure of modern philosophy to provide terminology with which to define and discuss the philosophical position he describes. The single word 'dualism' suffers from the fact that one of its several meanings is theological.

This problem was well recognized in the book *Science versus Materialism* (Methuen, 1940). The author, R.O. Kapp, professor of electrical engineering at University College London (with assistance from his colleague the professor of Greek), coined the term 'diathetics'. 'Diathesis' means the act of disposing to a specification or the being so disposed; a 'diatheme' is the result of a diathesis. These terms were included in *The 'Mental' and the 'Physical'* by H. Feigl (University of Minnesota Press, 1958), but have not gained currency.

On Kapp's view, the primary concern of your journal is non-diathetic reality — another expression for matter. But he would claim that in all that large fraction of your contents dealing with the structure and behaviour of living organisms you are also covering diathetics. In Zuckerkandl's words, such a view is 'naturalistic' but not in the least 'supernatural'. You would do a great service to the debate by adopting Kapp's terms almost 50 years after he proposed them.

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Letters submitted for Correspondence should be typed, double-spaced, on one side of the paper only. □

Splitting schizophrenia

Two papers in this issue, despite reporting apparently contradictory findings, pave the way for a genetic approach to the diagnosis of schizophrenia.

THE late US Supreme Court Justice Potter Stewart declared in a celebrated obscenity case¹ that, although he could not rigorously define pornography, "I know it when I see it". Psychiatrists are in much the same position concerning the diagnosis of schizophrenia. Some 80 years after the term was coined to describe a devastating condition involving a mental split among the functions of thought, emotion and behaviour, there remains no universally accepted definition of schizophrenia, no central feature at the heart of the disorder (such as mood change in manic depression) and no pathological underpinning (such as neurofibrillary tangles in Alzheimer's disease). Not surprisingly, psychiatrists have achieved no consensus on the aetiology of schizophrenia — although not owing to any shortage of theories. Accordingly, there will be substantial interest in the report of Sherrington *et al.* on page 164 of this issue² that schizophrenia in several Icelandic and English families is co-inherited with a particular region of human chromosome 5, indicating the presence of a gene, one form of which can confer a predisposition to schizophrenia. Lest one leap to the conclusion that the riddle of schizophrenia has been solved, however, Kennedy *et al.* report on page 167 of this issue³ that this same region of chromosome 5 is genetically unrelated to the incidence of schizophrenia in a large Swedish family, indicating (as psychiatrists had long suspected) that schizophrenia is a heterogeneous disorder with multiple causes. Together, these papers pave the way for a genetic approach to splitting schizophrenia into a collection of distinct diseases.

It has long been known that schizophrenia shows a tendency to cluster in families. In contrast to a 1 per cent lifetime risk of schizophrenia in the general population, the risk has been estimated at about 10 per cent for a first-degree relative of a schizophrenic and 50 per cent for an identical twin^{4,5}. Of course, familial clustering is not synonymous with genetic inheritance. Exposure of family members to a common environment has been suggested to play an important role, with agents such as slow viruses and psychological stress being implicated. A leading hypothesis in the 1960s identified the causative agent as overbearing mothers and asserted that identical twins were at special risk since schizophrenia involves a confusion of self-identity. In the

1970s genetic explanations gained favour, with transmission patterns in families being explained by increasingly sophisticated 'segregation analysis' (with, unfortunately, correspondingly decreasing ability to falsify the models as their complexity increased).

As with many fields of medicine, a major turning point in psychiatric genetics has occurred with the notion, first suggested by Botstein and colleagues⁶, of using common variations in DNA sequence as genetic markers to follow the inheritance of particular chromosomal regions in existing human families. Such differences can be conveniently detected when they disrupt the recognition site for a restriction enzyme, giving rise to restriction fragment length polymorphisms, or RFLPs. To date more than 3,000 RFLPs have been catalogued, and increasingly detailed genetic maps of the human genome have been assembled^{7,8}. By systematically testing RFLPs throughout the genome in families with such inherited diseases as cystic fibrosis and Huntington's disease, it has been possible to identify ones which display the same pattern of inheritance as the disease and thus must lie nearby the disease gene. In psychiatric genetics, the method has been used to detect linkage between DNA markers on chromosome 11p and manic depression in a single large Amish family⁹.

Although complete genomic searches will eventually be necessary for schizophrenia, Sherrington *et al.* and Kennedy *et al.* both decided to concentrate first on the chromosomal region 5q11–13. They were led to this region by a recent report¹⁰ of an uncle and nephew who both suffer from schizophrenia (as well as facial dysmorphologies) and who both carry an extra copy of this region translocated onto chromosome 1. On consulting their gene atlases, human geneticists noted that chromosome 5q contains the gene (GRL) encoding the glucocorticoid receptor, which seemed like a good candidate for a schizophrenia-susceptibility gene because perturbations in glucocorticoid metabolism can induce psychotic symptoms.

It is worth pointing out that this circumstantial evidence is alone quite flimsy. Inasmuch as various chromosomal abnormalities have been reported in schizophrenics, it is not unlikely that many gross genetic disruptions can non-specifically produce schizophrenia-like symptoms (just as schizophrenia can be phenocopied by such conditions as

temporal lobe epilepsy, brain injuries, early-stage Huntington's disease, amphetamine overdoses, thyroid disease and porphyria). As for the excitement about the GRL locus, subsequent studies by Kidd and colleagues¹¹ have revealed that it actually lies outside the 5q11–13 region. Undaunted, the two groups set out to test RFLPs from chromosome 5q in families with a high incidence of schizophrenia.

Sherrington *et al.* studied² five Icelandic and two English families, with 39 cases of schizophrenia (including all the main subtypes, such as paranoid and hebephrenic), 5 cases of related schizoid personality disorder and 10 cases of 'fringe' phenotypes not usually associated with schizophrenia (such as phobic disorder, anxiety disorder and major depressive disorder). Using two RFLPs to track the inheritance pattern in the 5q11–13 region, they find a strong concordance with the inheritance pattern of a putative dominant allele predisposing to schizophrenia. Interestingly, the concordance improved if the definition of affected cases was expanded to include the instances of schizoid personality disorder and the fringe phenotypes, suggesting that these may represent alternative clinical manifestations of the same allele. Lumping all cases together produces a lod score of 6.49, meaning that the inheritance pattern of the disease and markers was 10^{6.49}-fold more likely to have arisen if they are genetically linked than if they are unlinked.

Although newspaper reporters may now herald the isolation of 'the schizophrenia gene' (*Time* magazine some years ago announced the finding of "the math gene"), more sober readers will note the many caveats in the report of Sherrington *et al.*. First, and most important, the gene on chromosome 5 may represent only a minor cause of schizophrenia. In view of the complexity of schizophrenia, the investigators wisely biased their sampling of families to increase the prospect of detecting a single gene locus. By concentrating on families with a high proportion of schizophrenics, they enriched for rare alleles having very high penetrance. By focusing primarily on a small country such as Iceland, they may have enriched for a genetic isolate in which a usually rare allele has drifted to a higher frequency. Accordingly, the findings do not yet bear on the epidemiology of schizophrenia in the general population.

These caveats are not mere formalities,

as shown by the report of Kennedy *et al.*³ in this issue. Using an even more detailed genetic map (including five RFLPs on 5q), these investigators find strong evidence against linkage of schizophrenia in a single large Swedish family. The situation may turn out to be reminiscent of that for manic depression: the linkage to chromosome 11p detected⁹ in the large Amish family has, two years later, yet to be found in a second pedigree.

Second, the fringe phenotypes observed in the Icelandic and English families may not represent alternative manifestations of the schizophrenia-susceptibility allele. Only 5 of the 10 individuals were informative for linkage analysis (by virtue of the parent from whom they received the disease allele being heterozygous for the DNA markers tested) and so the evidence is not overwhelming. Further, each fringe phenotype occurs only once or twice and thus some may be purely coincidental; indeed, most cases of these fringe phenotypes in the general population are probably due to other causes.

Third, it would be wrong to draw the simplistic conclusion that all schizophrenia is entirely 'genetically determined'. Apart from the problem of heterogeneity, it is important to remember that the 50 per cent discordance rate among identical twins points to powerful non-genetic factors, which may be equally important to understand. The penetrance and expression of a schizophrenia-susceptibility allele is likely to be greatly influenced by environment.

Despite these cautions, the reports in this issue have broken the ice concerning the genetic mapping of schizophrenia. Sherrington *et al.* show for the first time that at least some cases of schizophrenia are apparently monogenic. Even if the locus on chromosome 5q does turn out to be a minor cause, it represents the first step in using genetics to subdivide patients into more homogeneous groups, which is likely to help the evaluation of potential therapies. Moreover, the finding that a single allele can apparently produce various subtypes of schizophrenia within a single family (paranoid, hebephrenic, schizoid personality, and so on) suggests that the psychiatric system of classification may not correspond to the underlying aetiology: ultimately, the study of variable manifestations of individual alleles may provide more reliable and useful diagnostic demarcations.

Whither now for psychiatric genetics?

• Further study of the Icelandic and English families is required. Because Sherrington *et al.* use only two modestly polymorphic RFLPs, nearly half of the meioses in their seven families are uninformative. By exploiting the increased power¹² of a detailed genetic map (as in Kennedy *et al.*³, or an even denser map) to double the available information, it will be

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The Indian Association for the Cultivation of Science.

Contents:

On the Mechanical Theory of the Vibrations of Bowled Strings and of Musical Instruments of the Violin Family, with Experimental Verification of the Results: Part I.
By C. V. Raman, M.A., Life-Member and Vice-President, Indian Association for the Cultivation of Science.

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C.V. Raman

29.6.1943

In memory of my visit to the Institute, to an authority on sound from an ignorant violinist? — a tribute from Yehudi Menuhin to C.V. Raman in 1952, on the occasion of the soloist's visit to the Raman Institute in Bangalore. See page 109 for extracts from Raman's papers on acoustics.

Yehudi Menuhin

June 11 1952



possible to determine whether all seven families show linkage: currently, the result rests primarily on evidence from two of the families. More detailed mapping will also help to localize the gene, whose position is only vaguely defined at present. Fortunately, the investigators plan to make available DNAs from immortalized lymphoblastoid cell lines (currently being derived) so that others may confirm and extend these mapping results using more detailed genetic maps (H. Gurling, personal communication).

• The Swedish family of Kennedy *et al.* will need to be studied with a complete RFLP linkage map to locate the susceptibility locus in this family, assuming that a monogenic aetiology exists. Pinpointing another locus will provide the most convincing proof of heterogeneity, because negative linkage results could in principle be caused by misdiagnosis. (The analysis by Kennedy *et al.* is relatively robust against failure to diagnose a true schizophrenic, but would be sensitive to incorrectly designating an unaffected individual as affected.)

• Similar RFLP studies must be carried out in many more affected families — both large pedigrees and smaller families with only 3 or 4 affected — both to test the 5q11–13 region and to locate additional genes predisposing to schizophrenia. In view of the apparent genetic heterogeneity, lod scores cannot simply be combined from independent families: special analytical methods must be used to extract the maximum information^{12,13}.

• Good genetics requires good phenotypes. Instead of concentrating solely on mapping schizophrenia *per se*, it may be fruitful to map the genetic basis for atypical biochemical or physiological responses found in some schizophrenics. Such traits have a number of advantages: they may represent more penetrant phenotypes of a schizophrenia-susceptibility allele (that is, they may be present in relatives unaffected with schizophrenia);

they may help distinguish among subtypes with different causes; and, being more easily and reliably assayed and possibly more penetrant, they should be easier to map. Once the genetic basis for such a trait is mapped, it can be correlated with the inheritance of schizophrenia.

Unlike much previous work aimed at finding biological correlates of schizophrenia, it is not necessary to find a trait which neatly distinguishes between schizophrenics and controls. Rather, one should search for traits that are enriched among schizophrenics and then test to see whether they also occur frequently in their relatives. As an example, amphetamine response is known to be extreme in about 30 per cent of schizophrenics and to show concordance between monozygotic twins: acute pharmacological challenge studies in relatives could clarify whether such response is a useful trait to study^{14,15}. Similar studies might be done with dopamine agonists and antagonists, since some of the latter are potent antipsychotic drugs.

In short, psychiatrists have now joined the ranks of experimental geneticists. To be successful in dissecting their particularly complex system, they will need to exploit the full range of tricks of that trade.

Eric S. Lander

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Evolution

Complex vertebrate structures

John Maynard Smith

It is a safe prediction that, from time to time, one of the less illiterate newspapers (the *Independent* is the most recent example; see illustration) will carry a centre-page article arguing that darwinism must be wrong because the structure of living organisms is too complex to have arisen by chance, and because anything other than a perfect structure would be useless. The orthodox reply is that we know of no other mechanism that could explain the evolution of adapted structure, that there is no quantitative difficulty in accounting for the accumulation of the necessary genetic information by selection, and that an imperfect structure is often not useless: an image-forming eye is fine, but an organ that tells you whether the light is on, or where it is coming from, may be a lot better than nothing, at least if you are a flatworm. Is there anything more useful that can be said? A recent meeting on the evolution of complex structures in vertebrates* attempted to answer this question.

Three groups discussed, respectively, the evolution of locomotion, of feeding, and of viviparity, and a fourth debated the problem of evolutionary mechanisms in more general terms. Did any general principles emerge from the many facts about vertebrate function that were presented? Two themes arose repeatedly — the significance of 'coupling', and the role of phylogenetic information in testing hypotheses. The term coupling was used in (at least) two senses: it meant either that the same function requires the cooperation of two or more structures or that the same structure performs two different functions. Little need be said about the former meaning: for example, the evolution of mammalian from reptilian locomotion required changes, not only in the skeleton and muscles, but in the nervous, vascular and respiratory systems. The necessity for simultaneous changes in all these systems may help to explain the extreme slowness of evolution, which is such a puzzle to those of us familiar with the results of artificial selection. But the other type of coupling — one structure performing two functions — has some less obvious consequences.

The general thesis is as follows. If a structure performs two functions, it is likely to be conservative in evolution. If, for any reason, it is released from one of those functions, this is often followed by rapid evolutionary specialization, and

sometimes diversification, for the other. An example will make this clear. In lung-breathing salamanders, the hyolingual skeleton is used as a pump to force air into the lungs. It is also used in feeding — in the aquatic larva to suck in water, and in the adult to propel the tongue in catching insects. However, loss of lungs has occurred repeatedly in salamanders, and in many cases this has been followed by the evolution of a mechanism propelling the tongue to a greater distance, with greater velocity and precision. But there is still coupling between feeding and breathing in the aquatic larva. In the *Bolitoglossa* salamanders the aquatic larval stage has

respiration, as do the inertial movements of the liver, which is rigidly attached to the diaphragm. Thus the birds have solved the problem by uncoupling: they use different structures for locomotion and breathing. The mammals continue to use some of the same structures for both purposes, but the evolution of new gaits, which involve flexing the backbone in a vertical rather than a lateral plane, has led to a system in which the two processes help rather than hinder one another.

Is there any way in which such ideas can be tested? How do we test the idea that lung loss is a cause of feeding specialization in salamanders, or that the acquisition of an additional and separately movable jaw apparatus in Cichlid fishes led to their adaptive radiation? The first requirement one must make of any such hypothesis is that it be plausible on engineering grounds. But often the comparative data enable us to go further. Suppose our

New answers to the scientific quarrels with Darwinism

William Rees-Mogg on the latest developments in the evolution debate

In 1967 I agreed with Maurice Macmillan an arrangement by which the *Journal of the Royal Society* would assist *The Times* with a joint scientific news service. Twenty years later, it has reported one of the most important scientific stories of the century, important, at any rate, if true. *Nature* is the most orthodox, and perhaps most authoritative, of scientific journals. The experiments reported in the current issue challenge one of the essential tenets of Darwinism.

"Darwinists believe in

now thought in their present condition to be only between 50,000 and 200,000 years old, there would be merely 2,500 to 10,000 generations of evolution back to that origin. Even after some millions of years, too few generations would have passed in the larger animals for a random process of genetic change to produce the perceived results.

"...second problem of Darwinism is that some

experiments conducted by Joshua Lederberg, that mutations do occur in bacteria which have never experienced the conditions under which such mutations would be beneficial.

These early experiments in bacterial genetics led most scientists to believe that random mutation was the sole origin of evolution. Now John Cairns and two colleagues at the Harvard School of Public Health have

ful, but will become so. This, as the *Nature-Times* news service reports, "contradicts the 'central dogma' of molecular biology: genetic information determines the character of an organism, but changes occurring in an organism cannot be transmitted back into its genetic make-up." It does not overthrow Darwinism as an important explanation of evolution — which is what Charles Darwin "has over"

novelty of the theory was not the idea of the survival of the fittest — the economists had understood that since Adam Smith, whose invisible hand operates by a process of competitive selection. The real novelty was the belief that the underlying process of evolution was random. That contradicted the belief — central to Thomist theology — that the universe showed design. Accident or design is the heart of the religious question.

This crucial difference between the religious and scientific

"Purposive response gives meaning to life. If *E. coli* has a purpose in life, so can the rest of us." (From the *Independent*, 13 September 1988.)

been lost, and further specialization of a high-speed projectile tongue has occurred: direct development has also made possible the evolution of stereopsis, needed to aim the tongue accurately.

A large-scale scheme based on the idea of coupling concerns the evolution of tetrapod locomotion. In a manner reminiscent of a recent US president, lizards cannot run and breathe at the same time, and hence are incapable of rapid sustained locomotion. This is because the rib-cage and associated musculature are used in both locomotion and breathing, but in different ways. The problem has been partially solved in the crocodiles by the evolution of an abdominal septum and diaphragmatic muscle used when breathing in. Birds can breathe and fly at the same time, but with different periodicities, so the two processes must be mechanically independent. This has been brought about by the evolution of a rigid rib-cage, of a rigid sternum to which the light muscles are attached, and of a novel machinery for driving air through the constant-volume lung. Mammals have taken a different road. In the canter, gallop and bipedal hop, there is a rigid 1:1 phase locking of gait and breathing. The locomotory muscles help to drive

hypothesis is that trait A is responsible for the evolution of trait B. If we have phylogenetic information in the form of a cladogram, we can ask the following questions. Is it true that the appearance of A preceded that of B? Has trait A arisen more than once, and, if so, was it always (or usually) followed by the appearance of B? The use of phylogenetic information in this way is still in its infancy, but, combined with appropriate statistical analysis of comparative data, it is spreading rapidly. This is admirable, but it is no substitute for engineering common sense. I do not need comparative data to tell me that a bird has a stout coracoid because, if it didn't, its shoulder joint would collapse onto its sternum every time its pectoral muscles contracted.

The group discussing evolutionary mechanisms was, inevitably, more argumentative than the others, and perhaps generated more heat than light. There was much discussion of development but, at a deep level, no real disagreement. No-one doubts that development limits the range of phenotypes that can arise in a particular taxon, and so constrains the course of evolution. What we need is more specific information about the nature of the constraints. ▶

* Dahlem workshop on complex organismal functions, integration and evolution in vertebrates, West Berlin, 28 August–2 September 1988. Proceedings to be published by Wiley.

The discussion of the role of 'species selection' was perhaps more illuminating. It was carried out in the presence of salamander biologists who insist that speciation often occurs without detectable morphological change, and of students of Cichlid fishes who insist that morphological change can occur without speciation. If the number of species in taxon A increases relative to the number in taxon B, this may be because of some property of individuals in taxon A — perhaps they run faster or chew better. Or it may be because of some property of the species in taxon A — perhaps they evolve faster, or speciate more frequently.

I would prefer to use the term 'species selection' only for the latter situation, and a more neutral term such as "species sorting" to refer to all cases of differential

survival or multiplication, however caused. But how important is species sorting anyway? My own view is that it is of trivial importance as a mechanism giving rise to the complex and adapted structures that were the subject of the conference. The reason for this is essentially quantitative. But species sorting may be of decisive importance in determining the distribution, in space and time, of different organisms. Because palaeontologists see dramatic changes in this distribution, but are often blind to adaptation, whereas naturalists see adaptation everywhere, but do not live long enough to be impressed by changes in taxonomic diversity, it is perhaps not surprising that they argue. □

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Sea-floor spreading

Feeling the pulse of the ridge

Joe Cann

CAN the pulse of sea-floor spreading at mid-ocean ridges be heard? Over the past year or two, marine geologists have been listening more and more closely. In three new papers, Harper¹, Norrell and Harper², and McCarthy *et al.*³ discuss evidence from the Josephine ophiolite complex in California, formed by Jurassic sea-floor spreading (more than 140 million years ago), and from old ocean crust in the Atlantic, instead of from the mid-ocean ridge axes, where most previous work has taken place. They all conclude that magmatic activity at spreading centres is episodic, and alternates with episodes of non-magmatic crustal stretching.

Idealized views of plate tectonics traditionally emphasize continuity, the global tape-recorder fossilizing the record of the Earth's magnetic field, the treacherous flow of mantle convection and the steady dribble of magma onto the ocean floor. This emphasis partly arises from the identification of episodic and cyclic models of the Earth with stasis: these are the models against which plate tectonics originally revolted. Certainly, the plates do, on a timescale of 10^4 – 10^5 years, move in a continuous way. But when the plate boundaries are examined on a smaller timescale, this picture fails. Recently, geologists have been looking more and more closely at the processes that create new ocean crust at mid-ocean ridges and find increasingly that episodic processes are significant.

At fast rates of sea-floor spreading, such as occur on the East Pacific Rise, the record of episodic spreading is clearly present⁴ but the effect becomes more striking at slowly spreading ridges. This is most apparent in the variety of morphologies of segments of the Mid-Atlantic

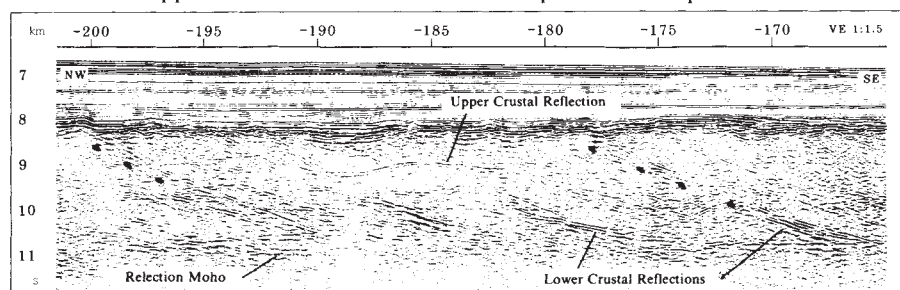
Ridge, spreading at 1 – 2 cm yr⁻¹ half rate⁵⁻⁷.

From these surveys it seems likely that there is some form of tectono-magmatic cycling in mid-ocean ridge processes. Sometimes sea-floor spreading would occur mostly by stretching and fracturing of already formed crust. At other times volcanism is copious, sea-floor spreading is by dyke intrusion and new lava flows create new upper ocean crust. In the

Complementing this is the evidence for faulting and thinning of the ophiolitic crust at an early stage in its evolution, with the faults soling out in a shear zone within the mantle. These faults would represent the phases of tectonic stretching.

McCarthy *et al.*³ present high-quality seismic reflection profiles from the old Atlantic crust east of Florida (see figure). There they are able to identify reflections from the Moho (which separates the crust and mantle), a discontinuous upper-crustal reflection which they interpret as coming from the boundary between sheeted dykes and gabbro, and reflections from normal faults mostly dipping towards the spreading centre. In addition they see strong lower-crustal reflections, dipping towards the spreading axis until they meet the Moho. These lower-crustal reflectors are 3–5 km long and dip at about 30°. These McCarthy *et al.* interpret as reflections from interbedded ultramafic and mafic rocks, representing cumulates that precipitated from primitive magmas as magma chambers were re-initiated after freezing. The periodicity of these reflectors would thus give an estimate of the rate of magmatic cycling.

How can this work be taken further? Detailed studies of slow-spreading ridges are still very rare. Surveys of other segments would enable a sequence of events to be established and for any cyclicity to be identified. No-one really expects perfect cycling: some crude alternation is more likely. But the causes of cyclicity and its consequences for plate tectonics will



Seismic cross-section between the West (NW) and Blake Spur (SE) fracture zones, south-west of Bermuda. The lower-crustal reflectors may be evidence of episodic magmatic cycling. (From ref. 3.)

spreading cell just south of the Kane Fracture Zone in the Atlantic, spreading currently involves a large volcanic ridge up to 500 m high and active hydrothermalism, but this is constructed on ocean crust which has been stretched and fractured, exposing deep structural levels on the walls of the median valley.

Harper¹ and Norrell and Harper² argue that two lines of evidence from the Josephine ophiolite support the notion of alternating volcanic and tectonic phases. The lava geochemistry shows a range from very primitive compositions to evolved iron and titanium-rich lavas in single lava sections, suggesting that magma chambers were initiated, evolved and solidified in the timescale of construction of the volcanic pile, and were thus highly episodic.

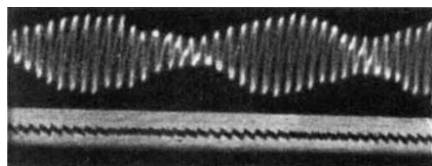
require more detailed coordinated and multi-disciplinary research. Such research is now being planned as a part of the evolving US RIDGE initiative and the parallel BRIDGE project in the United Kingdom. Other countries are expected to join these efforts to give a new international dimension to the solution of these important problems. □

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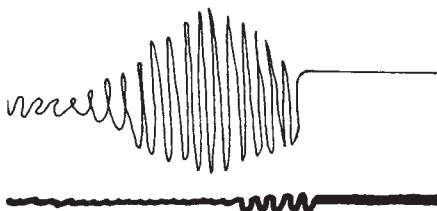
C.V. Raman on acoustics

In celebration of Raman's centenary, I have selected four extracts from his letters on acoustics, a topic to which he made significant contributions. Last week, I presented the first part of Raman's Letter on the wolf note of stringed instruments. In it he explained how, when the bow was pressed against the string with less than a critical force, energy requirements dictated that the fundamental mode could not be maintained and the motion passed over into one where the octave was dominant. This, in turn, could not be maintained and the original motion reverted. The excerpt below concludes Raman's argument.



The accompanying photograph showing the simultaneous vibration-curves of the belly [top] and string [bottom] of a cello amply confirms the foregoing explanation suggested by theory, and is itself of interest. It will be seen that the changes in the vibrational form of the string are about a quarter of a cycle in advance of those of the belly, and that in both curves the octave is conspicuous when the amplitude is a minimum.

In a subsequent letter Raman discussed the related effects in pizzicato.



The accompanying figure, showing the simultaneous vibration curves of the G string and bridge of a 'cello played pizzicato at the "wolf-note" pitch, presents some noteworthy features which may be of interest to readers of *Nature*. One of the striking features is the extremely rapid dissipation of energy. The other feature is the effect of the motion of the bridge on the vibration of the string. The figure may, in fact, be briefly described as showing a strongly damped coupled vibration of the string and bridge, in many respects differing from the cyclical vibrations, excited by bowing at the "wolf-note" pitch described by me in previous communications to *Nature*. At pitches slightly different from that of the "wolf-note", the dissipation of energy is far less rapid, and the motion of the string approximates to that of an ordinary damped harmonic vibration.

Raman's results remain the basis of discussion. Opinion is still divided between those who hold that the simple energy-input argument is all that is required and those, such as M.E. McIntyre of Cambridge University, who hold that the detailed nonlinear interaction between the bow and the string must be included in the analysis. This debate is touched upon in *Violin Acoustics* (ed. C. Hutchings) in the *Benchmark Series on Acoustics* (Dowden, Hutchinson & Ross, Stroudsburg, 1975).

J.M. Bowsher, University of Surrey.

Texts from *Nature* 97, 362 (29 June 1916) and 101, 264 (6 June 1918).

Nuclear physics

Hot nuclei and quantum liquids

Philip J. Siemens

ATOMIC nuclei are tiny droplets of immensely dense liquid. Fifty years ago, this realization first showed why and how nuclear fission releases energy when a large drop breaks into two smaller ones. Heiselberg, Pethick and Ravenhall¹ now extend this picture to explain the much more violent reactions that occur in the latest nuclear accelerators when nuclei moving near the speed of light smash into stationary nuclei, causing both the target and projectile to break into bits. They apply the modern theory of quantum Fermi liquids (such as liquid ³He) to these high-speed collisions, which had previously been described more crudely by analogy with classical liquids (such as liquid argon). Their work shows how further experimental and theoretical studies of these reactions can reveal the properties of matter at temperatures above 10¹¹ K and at densities around 10¹⁵ g cm⁻³, conditions otherwise occurring only in supernovae and during the first millisecond of the Big Bang.

The picture of the nucleus as a liquid drop is one of many macroscopic concepts that have proved useful for understanding nuclear phenomena (see box, page 110). Current work on nuclear collisions is especially exciting because it appears to be related to the dynamics of rapid phase transitions, a topic only partially understood even in large-scale practical applications such as laser annealing of materials. For nuclear collisions, in both the classical and the new quantum picture, the relevant phase transition is the passage from the dense liquid phase to a hot rarefied gas of nucleons or, if less heat energy is present, to a fog-like mixture of liquid and gas². Even if these phases are not actually produced in a collision, the existence of the phase transition causes instabilities which affect the outcome of the collision.

Collision

When two nuclei collide, the kinetic energy of the initial relative motion of the nuclei becomes available to excite other degrees of freedom, including the random thermal agitation of the nucleons as well as more organized collective modes of motion. Usually each of these modes is damped, so that the process eventually leads to the statistical equipartition of the available energy among the various degrees of freedom. But when the excitation energy and density enter the domain of the phase transition, some of the collective modes become unstable and thus can grow exponentially until they dominate the motion. The final stage of

the system will be determined mainly by the fastest-growing modes³.

For the liquid-gas phase transition, the unstable modes correspond to sound waves in the nuclear liquid. A sound wave can grow until the density in its rarefied regions becomes very small, at which point the matter is evidently broken into pieces. The size of the pieces reflects the wavelength of the unstable mode. Thus long-wavelength modes would produce a few large fragments, short-wavelength modes many smaller pieces.

Properties of sound

The outcome of a nuclear collision, then, depends on the properties of sound in nuclear matter: which wavelengths are unstable at various temperatures and densities, and how rapidly the unstable modes grow. At the beginning of the collision, the nuclear matter is in a liquid state, of higher density than the mixed phase or the nucleonic gas. As the thermal pressure of the hot matter causes it to expand, long-wavelength fluctuations become unstable at the 'spinodal' line $\partial P/\partial V = 0$ at which there is no impetus to uniformity; V and P are volume and pressure. Shorter-wavelength fluctuations require more energy to form the nascent surfaces⁴, and thus require lower densities to be able to grow. But shorter waves generally correspond to faster motion than longer waves, and thus can grow faster once they become unstable⁵.

The region of dynamic instability, defined by the spinodal line, is sensitive to the quantum nature of the nuclear liquid. In a degenerate Fermi fluid, in which all the lowest quantum states are occupied, the Pauli principle inhibits the collisions of nucleons by preventing them from scattering into the already-occupied quantum states. (Exactly this effect is responsible for the long mean free paths of electrons in pure metallic crystals.) In the absence of collisions, the appropriate spinodal curve is isothermal, because the long mean free path enhances the thermal conductivity; in the collision-dominated hydrodynamics of classical fluids, on the other hand, the instability sets in at the adiabatic (isentropic) spinodal, which occurs at lower density. In their new work, Heiselberg, Pethick and Ravenhall¹ estimate that, at the temperatures and densities of interest, the mean free path of nucleons in nuclear matter is larger than even the largest nuclei. Thus breaking up nuclear matter requires a greater density excursion than would be necessary in a classical fluid with the same equation of state. ▶

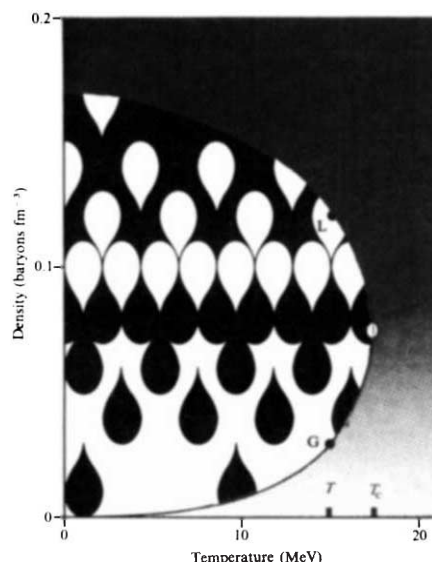
Macroscopic behaviour of nuclear matter

The quantum revolution began with the insight that many classical concepts used to describe macroscopic objects break down when applied to atoms and molecules. Although the replacement of classical descriptions by quantum concepts was thought necessary because of the atoms' microscopic dimensions, several other factors can also determine the importance of quantum effects. Occasionally quantum phenomena appear on a macroscopic scale: superconductivity is an important example, but another is low-temperature helium, which remains liquid even at absolute zero instead of solidifying as a classical liquid would. Conversely, typical classical behaviour is well documented in atomic nuclei, even though they are 10^{-4} times smaller than the atoms in which they reside. The following are some of the remarkable macroscopic features of nuclei.

Density of nuclear matter. The volume of each nucleus is proportional to the number of nucleons it contains: each nucleon requires about $6 \times 10^{-39} \text{ cm}^3$. This uniformity is typical of macroscopic materials; by contrast, atoms are all about the same size whether they contain one electron or 100.

Binding energies. The energy that holds a nucleus together is nearly proportional to the number of constituent nucleons, with corrections for the reduced binding of the nucleons on the surface and for the electrostatic repulsion of the protons. Uniform macroscopic systems share this feature, whereas the binding energy of atoms increases much more rapidly with the number of electrons.

Statistical mechanics. Because the volume and binding energy of a nucleus are



Schematic phase diagram for nuclear matter. Open circle, critical point. G and L (which lie on the spinodal curve) illustrate gas and liquid densities, respectively, for a temperature T near the critical temperature T_c . (From ref. 2.)

proportional to the number of nucleons it contains, we may extrapolate from finite nuclei to the thermodynamic limit of infinitely extensive matter. The standard methods of quantum statistical mechanics permit the definition of a nucleus's entropy via its density of quantum states. When the excitation energy is large enough to eject a nucleon from the nucleus, it is not even necessary to introduce an artificial coarse-graining: a natural energy smearing is provided by the finite lifetime of the nucleus attributable to the emission of nucleons, which is known as evaporation. The concept of temperature can also be

introduced via its canonical relation to the energy and entropy.

Phase transitions. Even though nuclei have no more than a few hundred nucleons, the interplay between the free energy of a low-density gas and the short-range attractive forces that bind the nuclear liquid leads to the possibility of a liquid-gas phase transition for the same reasons as in a classical van der Waals fluid. In a finite system the phase boundaries are not sharp, but nevertheless quite distinct as is well known from molecular-dynamics simulations of classical fluids. The long-range electrostatic forces in nuclei complicate the phase transition but do not eliminate it.

Collective oscillations. Just like a droplet of compressible classical fluid, nuclei exhibit several kinds of collective oscillations. Well documented examples include volume-conserving vibrations of the drop's shape, standing waves of density inside the nucleus, spin waves and even 'isospin' oscillations in which neutrons and protons move oppositely.

Irreversible dissipative motion. Collective oscillations whose energy exceeds that necessary to evaporate a nucleon are damped by their coupling to the heat bath of statistical excitations of the nucleus; true irreversibility is manifest without artificial coarse-graining.

Superconductivity. The ground states of nuclei are dominated by Cooper pairs of nucleons moving in time-reversed quantum states, in close analogy with the BCS picture of superconductivity. The extensive evidence for this nuclear phenomenon is primarily related to the existence of the pairs: no one has yet measured the resistance of a nucleus. P.J.S.

The rate at which density fluctuations grow in nuclear matter is also affected by the quantum suppression of collisions. Heiselberg, Pethick and Ravenhall¹ obtain the first quantitative estimates of the growth of density instabilities in expanding hot nuclei by applying the Landau theory of degenerate Fermi liquids, which was developed² for liquid helium. They take the parameters of the Landau effective interaction from previous studies of the single-particle and collective excitations of cold nuclei. Linearization of Landau's kinetic equation leads to a simple estimate of the growth rate of the unstable modes. Using these growth rates together with simple estimates of the rate of expansion of the hot nuclear matter, they can predict which initial conditions of temperature and density will permit each mode to grow enough to break up the matter. As expected, the short-wavelength modes grow most rapidly; if all modes are randomly excited, the

shortest wavelengths will prevail (of course, only modes with wavelengths larger than the internucleon spacing are considered). The authors point out, however, that the initial conditions of a nuclear collision are not random, but may imply the early preferential excitation of long-wavelength modes.

The application of the known theory of quantum liquids to violent nuclear collisions points the way to a quantitative interpretation of current experiments. Recently commissioned accelerators in France (GANIL, 1987) and the United States (Michigan State, 1988) permit for the first time a systematic determination of the results of nuclear collisions in the range of projectile velocities around half the speed of light, where the effects of the liquid-gas phase transition should be most prominent. The experimental programme is very demanding, because the apparatus must be constructed to identify simultaneously a hundred or more reaction

products with widely-varying masses and speeds. An equally ambitious theoretical programme is needed to extend the techniques pioneered by Heiselberg, Pethick and Ravenhall to include both the highly ordered motion at the beginning of the collisions and the non-linear evolution of the instabilities, as well as to produce detailed predictions of the many-fragment final states. The promised reward for this effort is a terrestrial glimpse at the properties of matter under extreme conditions characteristic of the most extravagant astrophysical phenomena. □

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Stellar astronomy

Life and death above the plane

Virginia Trimble

MASSIVE, short-lived stars have no business being located hundreds or thousands of parsecs out of the galactic plane, for the giant molecular clouds from which they normally form are confined to a layer with a scale height of 50–70 parsecs, and young stars, as a rule, move slowly. We have, nevertheless, known of a few counterexamples for more than 30 years¹. A fistful of recent papers^{2–10} casts light of varying colours on the phenomenon and forces us to the conclusion that these aberrant objects are not all the same kind of thing. Every hypothesis so far suggested (and perhaps a few yet to be formulated) probably applies somewhere.

The outlaw stars include what appear to be hot (O, B and A), main-sequence (core hydrogen burning) stars with expected lifetimes of 10^6 – 10^7 years and cooler (A–F) supergiant (shell hydrogen or helium burning) stars that are normally descendants of OB main-sequence stars, and so also short-lived. What are we supposed to make of them?

First, the stars might really be old ones, passing through an evolutionary phase that mimics young main-sequence stars and their progeny. Then, the normal increase with age of stellar velocity dispersion and scale height would entitle the stars to their present position. Second, they might be fainter than normal stars of similar colour and surface gravity. Then their distances will have been overestimated and their positions will not be excessively far from the galactic plane. These two can operate together in the case, for instance, of OB subdwarfs which are both much fainter than OB main-sequence stars and much older, being the last pre-white-dwarf gasp of stars with billion-year-plus lifetimes, like our Sun.

Third, something could have greatly delayed the completion of core hydrogen burning. Fourth, the stars might have formed as usual in the galactic plane but acquired anomalous velocities large enough to carry them to their present positions through disruption of binary systems or parent clusters. And, fifth, formation of massive stars may occasionally occur outside the galactic plane.

Consequences

Most of these ideas have observable consequences: sufficiently old stars should be metal poor; velocities are measurable (at least radial velocities); and so, sometimes, are masses. It is along these lines of observational tests that most of the recent progress has occurred. The high latitude A–F supergiants, whose prototype 89

Her has been with us since 1951 (ref. 11), turn out largely to be examples of the first hypothesis. They are systematically metal-deficient^{2–4}, especially in s-process elements like barium, and so almost certainly more than about 10^{10} years old. The most likely evolutionary state for them is the brief proto-planetary-nebula phase, between the termination of helium-shell burning and illumination of a true planetary nebula. Stars of less than 1 solar mass take more than 10^{10} years to get this far.

The high-latitude main-sequence A stars¹², on the other hand, seem to be experiencing prolonged core hydrogen burning. A few such stars occur in open and globular clusters, where they are called blue stragglers because of their location on a graph of luminosity versus colour (Hertzsprung–Russell diagram). It may not seem much progress to have reduced one mysterious phenomenon to a special case of another equally mysterious phenomenon. But the number of blue stragglers released as clusters disperse is, at any rate, sufficient to account for the high-latitude A stars (ref. 10). The main-sequence phase can be lengthened either by mixing fresh hydrogen down into the core of a single star or by bringing in more fuel from a second star in a binary system or collision. There is observational evidence for mixing in the form of enhanced nitrogen in blue stragglers in young associations¹³ and for transfer or mergers in the form of anomalously high masses for the blue stragglers in the globular cluster NGC5466 (ref. 14). Mergers are bound to occur in the crowded conditions of cluster cores¹⁵.

OB stars that are really old, faint subdwarfs often give away the fact by displaying high surface gravities. About 200 such stars have been catalogued¹⁶. For others, we can probe the mass (hence luminosity, using the spectroscopically determined

surface gravities and temperatures) indirectly. Some are probably faint; others almost certainly not.

Ultraviolet spectra of RWT152, for instance¹⁷, reveal a wind whose velocity implies an underlying star of only 0.6 solar masses, and so subdwarf luminosity, despite the apparently normal optical spectrum. On the other hand, PHL346 (ref. 6) and several other high-latitude OBs (ref. 5) display variable brightness and radial velocity of the sort whose prototype is β Cephei. If the variability has been correctly interpreted as non-radial modes, then the periods imply masses near 10 solar masses and lifetimes of 10^7 years. Other stars show such exceedingly normal line ratios for their temperatures and gravities⁷ that it is very hard not to interpret them also as massive main sequence stars. Thus we really do find short-lived objects 1–5 kpc above the galactic plane.

Runaway stars

How did they get there — ejection from the plane (and, if so, how?) or formation *in situ*? Classical runaway stars¹⁸ are OBs whose three-dimensional velocity vectors point back to young associations within which they probably formed. We cannot have this information for stars many kiloparsecs from the galactic plane because their motions in the sky will be immeasurably small. But for the ones at high latitude, motion out of the plane is largely along our line of sight and the measured radial velocities (up to 250 km s^{-1}) are frequently large enough to have carried the stars from plane to present position in the available time^{7,8,19,20}. The ejection mechanism is violent enough to disrupt wide binary systems, because visual binaries are common among OB cluster members, rare among field stars, and non-existent among runaways²¹.

Two candidate accelerators have been suggested — a supernova explosion of a close binary companion or close encounters in the cluster of formation. The former presents several difficulties. Owing to mass-transfer effects, a symmetric explosion leaves the secondary either with the neutron star still bound to it or

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with a velocity of at most 35–40 km s⁻¹ (ref. 22); and most OB runaways do not have neutron star companions²³. Cluster ejection, on the other hand, does seem capable of expelling both single stars and close pairs at the observed speeds⁹.

Finally, there are a few OB stars like PHL346 (refs 6, 23) that appear to be genuinely young and far from the galactic plane and yet to have velocities far too small or even in the wrong direction to get them there. What evidence do we have that they could or could not have formed where we see them? Gas clouds exist up to several kiloparsecs from the plane^{24,25} and collisions between them could plausibly

trigger star formation²⁶. The process must, however, be quite rare and localized, in the sense that the high-latitude clouds presently known to be forming B stars^{27,28} are nearly all within 200 parsecs of the plane. Admittedly the techniques used to find them might not pick out more distant examples. Thus we still cannot be quite certain whether stellar life and death above the galactic plane is preceded by birth there. □

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Genetic code

Modified bases and aminoacylation

Uttam L. RajBhandary

THE discovery of modified bases in the transfer RNAs of all organisms has stimulated much interest in their role¹⁻⁴. Until now, it has been thought that the main role of base modifications in tRNA is in modulation of translational efficiency and/or codon specificity⁵. Base modifications have not been thought to be involved in specificity of aminoacylation, that is, attachment of the correct amino acid to the correct RNA. Now Muramatsu and co-workers report on page 179 of this issue⁶ a striking result in which a particular base modification in an *Escherichia coli* isoleucine tRNA is important for reading the AUA isoleucine codon and for the specificity of its aminoacylation.

The dual effect of a single base modification in specificity of aminoacylation and in codon reading highlights a feature of tRNA research that has generated much recent interest. The ability to mutagenize tRNA genes and to study mutant tRNAs have led to significant advances in understanding the relationship between the structure and function of tRNAs^{6,7}, in particular, identification of sequence elements important for aminoacyl-tRNA synthetase recognition⁸⁻¹³. Such studies have reinforced and extended earlier work which suggested that simple changes in a tRNA can sometimes have striking effects on its properties. Some of these results have generated much excitement in the field and have been the subject of recent commentaries¹⁴⁻¹⁶.

It was already known that modified bases have both subtle and well-defined roles. *E. coli* mutants lacking ribothymidine can grow normally, for example, with only a slight reduction in growth rate of about 4 per cent³. But this deficiency in a tRNA facilitates initiation of protein synthesis without formylation of the initiator tRNA in *Streptococcus faecalis* and in some *E. coli* mutants^{17,18}. The role of modified

bases located in and around the anticodon sequence of tRNA is much clearer; base modifications here increase translational efficiency of the tRNA and fidelity of the translation process³. Mutations in genes encoding some tRNA base-modifying enzymes give rise to effects such as the derepression of amino-acid biosynthetic operons, many of which are regulated by transcriptional attenuation¹⁹.

E. coli contains two isoleucine tRNA species, a main species with GAU as its anticodon, which reads the AUU and AUC codons, and a minor species with LAU as its anticodon, which reads the remaining isoleucine codon AUA. Lysidine (L), a modified base derived from cytosine in which the 2-keto group is replaced by the amino acid lysine, is thought to pair only with A, allowing the tRNA with LAU in the anticodon to read the AUA codon for isoleucine.

Muramatsu *et al.* in their new work⁶ used RNA excision and ligation techniques to replace the anticodon LAU with CAU. They show that the mutant tRNA, with the single change of L to C, is a much poorer substrate for *E. coli* isoleucyl-tRNA synthetase (IleRS). Interestingly, it is now a very good substrate for *E. coli* methionyl-tRNA synthetase (MetRS). These results establish a clear role for a post-transcriptional modification of tRNA in determining aminoacylation specificity and demonstrate the critical role of the anticodon sequence in discrimination between tRNAs by IleRS and by MetRS.

This result supports the earlier conclusion that the most critical requirement for MetRS recognition of a tRNA is the anticodon sequence CAU. Switching of UAC in the anticodon of valine tRNA to CAU has now been shown² to lead to loss of valine acceptance, and to gain of methionine acceptance. Conversely, switching

of the methionine tRNA anticodon from CAU to a valine anticodon, UAC, abolishes methionine acceptance. However, the mutant tRNA is now aminoacylated with valine by ValRS. Taken together^{8,14,16,20}, these results suggest that for MetRS, ValRS, IleRS and some others, the anticodon sequences in the cognate tRNAs constitute the main specificity-determining contacts. The lack of aminoacylation of isoleucine tRNA by MetRS probably results from the base modification L in the anticodon of isoleucine tRNA preventing MetRS from making effective contact with this base, which is critical for recognition.

Replacement of L 34 by C 34 in isoleucine tRNA leads to a substantial reduction in the rate of aminoacylation with isoleucine. This implies that L provides an important contact site for IleRS. Because the main isoleucine tRNA of *E. coli* has a guanosine (G) at this position, IleRS could contact functional group(s) common to both G and L. An alternative possibility, favoured by Muramatsu *et al.*, is that C 34 constitutes a "negative determinant" for IleRS, that is, presence of C 34 somehow interferes with IleRS recognition of this tRNA. These suggestions could be tested by studying the effect of replacing L by U or A on IleRS recognition.

The finding that the presence or absence of a single base modification can have such dramatic effects on properties of a given tRNA does not mean that other base modifications have a similar effect on other tRNAs. Several tRNAs lacking all base modifications have now been prepared using T7 RNA polymerase^{21,22}, and show only small differences in aminoacylation kinetics compared with tRNAs carrying the full complement of base modifications.

Change of L to C affects the coding specificity from AUA (isoleucine) to AUG (methionine) and aminoacylation specificity also, from isoleucine to methionine. (The possibility that the mutant

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isoleucine tRNA with CAU anticodon also reads AUA has not been rigorously tested.) If the mutant tRNA reads exclusively the AUG methionine codon, this would be a simple mechanism for *E. coli* to minimize translational errors arising from partial modifications. If modification of C to L is incomplete, tRNA that

contains an unmodified C would be aminoacylated with methionine and read the methionine codon; tRNA that contains L would be aminoacylated with isoleucine and read the isoleucine codon. □

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Calcium channels

Diversity and complexity

Harald Reuter and Hartmut Porzig

RESEARCH on calcium channels in cell membranes has escalated dramatically in the past 10 years. The concentration of calcium ions within and between cells is in part regulated by voltage-gated ion channels, which are proteins embedded in the cell surface membranes. Calcium channels have attracted the attention of electrophysiologists, biochemists, molecular biologists and clinicians, who recently met* to discuss how the channels are regulated. When discussing these channels, the difficulty starts with the terminology of their classification and goes on with a multitude of factors which modulate their behaviour. A simplified scheme of three main groups of calcium channels is shown in the figure. These groups probably should be divided up further as our knowledge of their electrophysiological and molecular properties, their functional significance and their modulation by hormones, neurotransmitters and drugs expands.

Electrophysiology

When should an ion channel be called a calcium channel? The only criterion is whether under physiological conditions an open channel has a high selectivity for passing Ca^{2+} ions. This does not mean that under experimental conditions the current flowing through the channel cannot be carried by other cations. The high calcium selectivity ($\text{Ca}^{2+}:\text{Na}^{+}, \text{K}^{+} > 1,000:1$) of the channels seems to result from ion-channel as well as from ion-ion interactions (P. Hess, Harvard Medical School, Boston; P. Kostyuk, Bogomoletz Institute, Kiev; D. Lux, Max Planck Institute, Munich). Ca^{2+} ions at micromolar concentrations effectively block the flow of monovalent cations through the channel by occupying with high affinity a binding site within the permeation pathway and by allosterically influencing cation binding at other sites of the protein. Permeation of calcium through the open pore is thought to be achieved by electrostatic ion-ion repulsion in the single-file permeation pathway^{1,2}. Only two types of calcium channels (T

and L) have so far been fully analysed in terms of their ion selectivity.

Local fluctuation of the cytosolic Ca^{2+} concentration depends to some extent on the amount of Ca^{2+} that flows into the cell during the opening of calcium channels. Different types of channels seem to be involved in different cellular functions that depend on such local fluctuations. For example, T-type calcium currents influence sinoatrial pacemaker activity in the heart, whereas L-type channels help maintain its contractility and, in some neurons, N-type channels participate in neurotransmitter release (R. W. Tsien, Yale University). Therefore, it is not surprising that considerable effort is being directed towards understanding the mechanisms involved in modulation of calcium-channel activity.

An arsenal of transmitters, hormones, toxins and drugs has meanwhile been

discovered to influence calcium-channel gating. Voltage-dependent effects on calcium-channel kinetics of various neurotransmitters have been analysed by measurement of gating currents (charge movements associated with channel openings; B. Bean, Harvard Medical School). Agonists of β -adrenoceptors, via a phosphorylation reaction³, cause shifts of gating currents towards more negative potentials and, thereby, enhance L-type calcium currents much more at moderate than at strong depolarizations. The opposite is true for α_2 -adrenoceptor stimulation in dorsal root ganglion cells, where N-type calcium currents are reduced. Dopamine slows activation of putative L-type channels and inhibits the current flowing through T-type calcium channels in sensory neurons, effects that can be mimicked by intracellular application of GTP- γ -S (E. Carbone, University of Torino). Similar results have also been reported for other substances (somatostatin, dynorphin) and indicate an involvement of G-proteins in calcium-channel gating. G-protein, or its α -subunit, has not only been shown to modulate L-type currents in cardiac and skeletal muscle, but also to prevent run-down and maintain channel activity in excised inside-out patches of membrane (A. M. Brown, Baylor University, Houston).

Molecular biology

Tanabe *et al.* recently reported⁴ the primary structure of the dihydropyridine receptor from rabbit skeletal muscle

Muscle disease and molecular cloning

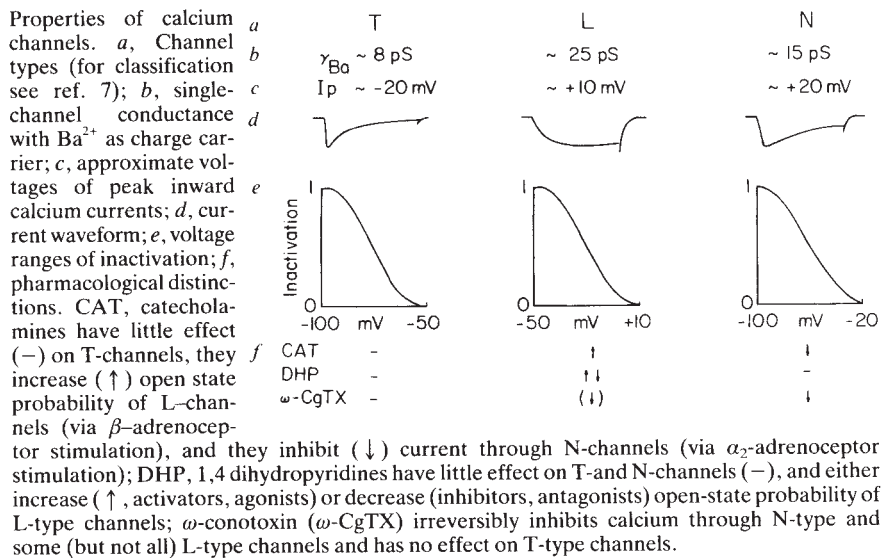
LAST year⁴, Numa's group elucidated the primary structure of the 1,4-dihydropyridine (DHP) receptor (α_1 -subunit of the DHP receptor complex) from skeletal muscle by cloning and sequence analysis of DNA complementary to its messenger RNA. These results suggested that this receptor, located in transverse tubular membranes, is both a voltage sensor for excitation-contraction (EC) coupling and a calcium channel. But it was not then possible to obtain a functional calcium channel by corresponding cDNA expression studies in *Xenopus* oocytes. On page 134 of this issue⁵, Numa's group, in collaboration with Beam, now report the success of this strategy.

In cultured skeletal muscle cells from mice with muscular dysgenesis, these authors were able to restore EC coupling and the slow calcium current by microinjection of an expression plasmid (pCAC6) carrying the DHP receptor cDNA. They first had to identify the genetic defect that leads to muscular dysgenesis. This autosomal, recessive trait is characterized by failure of EC coupling and lack of a low calcium current in skeletal muscle, but

not in the heart. The authors then analysed the genomic DNA of normal mice and of animals that were homozygous or heterozygous for the muscular dysgenesis mutation.

In diseased mice, they find a difference in two regions of the structural gene encoding the DHP receptor, resulting in a large reduction of the corresponding messenger RNA. Microinjection of the relevant expression plasmid into nuclei of cultured myotubes from dysgenic mice restores spontaneous or electrically evoked contractions and DHP-sensitive slow calcium currents. The contractions are independent of the flow of the slow calcium current, indicating that the DHP receptor has two functions: that of the voltage-sensing system involved in EC coupling and that of a calcium channel. Although the number of successfully injected myotubes was rather low in these experiments, the results are completely convincing. They herald the use of a new vertebrate expression system in which a structurally defective gene has been identified, corrected and translated into a functional membrane protein. H.R. & H.P.

*Calcium channels: Structure and Function, organized by the New York Academy of Sciences at the Royal Society, London, 18-20 July 1988.



deduced from its complementary DNA sequence. Although they suggested that this polypeptide ($M_r=170\text{K}$) might be both the voltage sensor for excitation-contraction coupling as well as a calcium channel, this suggestion was difficult to prove. A collaboration between S. Numa's group (Kyoto University) and K. Beam's group (Colorado State University, Fort Collins) has now been successful. They microinjected a cDNA carrying an expression plasmid into cultured skeletal muscle cells from mice with muscular dysgenesis, which lack the messenger RNA for excitation-contraction coupling and L-type calcium channels. The protein encoded by the cDNA restores both contractility and calcium currents — see their paper on page 134 of this issue⁵ and the box on page 113. But it is not clear whether other proteins which copurify with the 170K polypeptide (see below) are present in muscle from dysgenic mice and whether they participate in the functional properties of calcium channels. The first hint that the 170K subunit (α_1) of the dihydropyridine receptor complex may be sufficient to serve as a functional calcium channel comes from reconstitution experiments in lipid bilayers (D. Pelzer, Universität des Saarlandes, Homburg/Saar), although the success rate of these experiments seems to be very low.

Biochemistry and immunology

At least three additional polypeptides from skeletal muscle T-tubules copurify with the α_1 -protein: α_2 (~145K), β (~50K) and γ (~30K). They form a 1:1:1:1 complex which has a M_r of ~400K (W. Catterall, University of Washington, Seattle; H. Glossman, University of Innsbruck). The main evidence for the existence of a functional complex with this composition comes from studies with antibodies against each of the four polypeptides (K. Campbell, University

of Iowa). Specific antibodies against α_1 and γ both immunoprecipitate all four subunits. Crossreactivity patterns suggest that the small β - and γ -subunits are not fragments of the two larger components. Moreover, antibodies against α_1 (ref. 6) and against β and γ components modulate L-channel function. Anti- β enhances calcium currents and prevents the inhibitory action of nitrendipine, whereas anti- γ inhibits channel activity.

There is considerable immunological heterogeneity even among L-type channels from different tissues and species. Antibodies against α_1 seem to be tissue-specific, whereas those against the α_2 subunit show fairly wide crossreactivity (M. Hosey, Chicago Medical School; R. Norman, University of Leicester). Similarly, a naturally occurring specific auto-antibody against L-type channels of presynaptic nerve terminals in the neuromuscular endplate associated with Lambert-Eaton syndrome shows little crossreactivity with L-channels in other tissues (D. W. Wray, Royal Free Hospital, London). These observations open new prospects for the therapeutic modulation of channel function by more specific drugs. Information as detailed as that available for L-type channels in skeletal muscle is still lacking for calcium channels expressed in other tissues. Clearly, the calcium wave has not yet ebbed. □

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Daedalus

Lighten our darkness

ARTIFICIAL lighting is ridiculously inefficient. Of a million photons thrown out by the ferocious lights of a TV studio, for example, perhaps only one will ultimately enter a camera lens; all the rest are wasted. So Daedalus is devising a camera which probes the scene with its own photons, and gathers them back again. A lamp on the camera shines into an angled beam-splitter in front of the lens, launching the light at the scene as if it came from the lens. By itself, this has almost no advantages. An illuminated object bounces very little light back to its source; most is scattered in all directions. But Daedalus will paper his studio with special reflectors which intercept these scattered photons and throw them back exactly where they came from. Each will return to the illuminated object exactly at the point from which it was originally scattered. Now any optical path is perfectly reversible. So the returning photon will precisely retrace its previous scattering, and will be launched back into the camera lens from which it came. Thus most of the incident light, not merely one part per million, will go to form the image.

The crucial elements of this scheme are, of course, the special reflectors. They should ideally be phase-conjugate mirrors, which reverse a photon's path exactly. Sadly, such mirrors require elaborate laser technology and only work for specific wavelengths of light. So Daedalus will rely on the much simpler cube-corner reflectors, in the form of large plastic sheets embossed with innumerable closely packed cube-corner indentations, and aluminized for high reflectivity. Most photons hitting such a sheet will bounce straight back where they came from.

Expensive and wasteful TV lighting will become a thing of the past. The tiny lamp on each camera, its light perfectly utilized, will do it all. Furthermore, each returning photon will have been scattered twice by the same point of the scene — once on its outward journey, and once on its return. So dark or coloured areas will imprint their absorption spectrum on the light with double efficiency. Contrast and colour saturation will be dramatically enhanced. The source of light in the TV picture will appear to be the reflecting sheets — light hitting them will come brightly back to the camera, while regions blocked off from the reflectors will return no photons and will seem in deep shadow. Since the reflectors can cover much of the ceiling and walls of the studio, wonderfully even and shadowless lighting will be possible. Some conventional lighting will still be needed, however, to pierce the new efficient gloom and let the actors and crew see what they are doing.

David Jones

Characterization of a seal morbillivirus

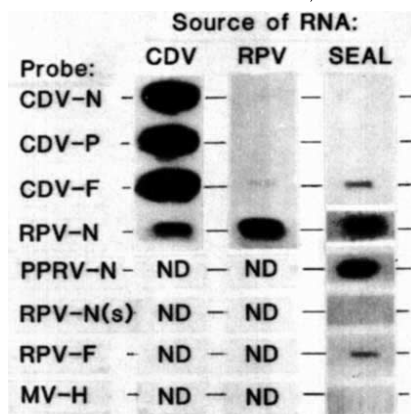
SIR—Recent reports have provided evidence that canine distemper virus (CDV) may be the cause of the recent epizootic of seal deaths in the Baltic and North Sea¹⁻³. CDV is one of four known viruses which form the morbillivirus genus of the family Paramyxoviridae. The other three are measles (MV), affecting man, peste des petits ruminants (PPRV) affecting small ruminants and rinderpest (RPV) affecting all artiodactyls. The four viruses show close serological relationships⁴, and there is nucleotide sequence homology between the genes encoding the principal immunizing protein, the fusion protein (F), of CDV, MV and RPV^{5,6}. Thus it is difficult to distinguish, for example, CDV from RPV infection on the basis of serological evidence unless specific monoclonal sera are used¹.

Recently, we have prepared DNA clones of a number of morbillivirus genes, and have developed a specific diagnostic test, based on DNA-RNA hybridization, for analysis of post-mortem specimens. The test distinguishes RPV from PPRV and other morbillivirus infections⁷. The method was effective in confirming that a recent outbreak of cattle plague in Sri Lanka, the first for forty years, was RPV and not PPRV, although the disease was apparently introduced by sub-clinically infected goats from India. We used the DNA probes to examine seal spleen tissue supplied by the Sea Mammal Research Unit, Cambridge. The material came from a common seal (*Phoca vitulina*) that had died on the east coast of England.

RNA was extracted using guanidinium thiocyanate from 2 ml of chopped spleen tissue from the affected seal, blotted onto Hybond N membrane, and hybridized under stringent RNA/DNA conditions to complementary DNA probes as described elsewhere⁷.

No hybridization could be detected between RNA from the diseased seal and probe DNA copies of the nucleoprotein (N), or phosphoprotein (P) genes of CDV (see figure). Low levels of hybridization to seal RNA were seen with the F genes of CDV and RPV, which cross-react between themselves and different morbilliviruses. These data suggest the presence of RNA from a morbillivirus but not CDV. Of the N-gene probes tested, only full-length N genes of RPV and PPRV gave positive hybridization to the seal RNA confirming some relationship to these two morbilliviruses. However, two pieces of evidence suggest that the virus is not RPV itself. First, when we used a short N-gene probe, RPV N(s), which contains only 30% of the N-gene sequences of RPV, no hybridization was detected (see figure). Second, as noted above, equivalent hybridization was seen using

the N-gene probe of PPRV. The RPV and PPRV N-gene probes have been tested on 16 strains of RPV and six strains of PPRV⁷ and have been shown to distinguish the viruses unequivocally. This suggests that the virus present in the spleen of the diseased seal is neither RPV nor PPRV. In addition, the CDV N gene does not cross-hybridize with RNA from other known morbilliviruses. Thus, it is likely



Hybridization of membrane-bound RNA isolated from CDV-infected Vero cells, RPV-infected Vero cells, and spleen tissue from a diseased seal with various cloned cDNA probes of morbillivirus genes. The probes were labelled with [³²P]-dATP by the random priming method and hybridization was detected by autoradiography⁷. N, nucleoprotein; P, phosphoprotein; F, fusion protein; H, haemagglutinin; N(s), fragment representing about 30% of the nucleoprotein gene; ND, not determined. The level of hybridization of the CDV-derived N probe to CDV-infected Vero cell RNA was higher than the RPV-derived N probe to RPV-infected Vero cell RNA because the Vero cell-adapted CDV strain replicates to higher titre in Vero cells than does RPV.

that a new morbillivirus is associated with the seal deaths. We are currently isolating the morbillivirus genes by cloning and selection of RNA from infected seal tissues, so that nucleotide sequence analysis can be performed.

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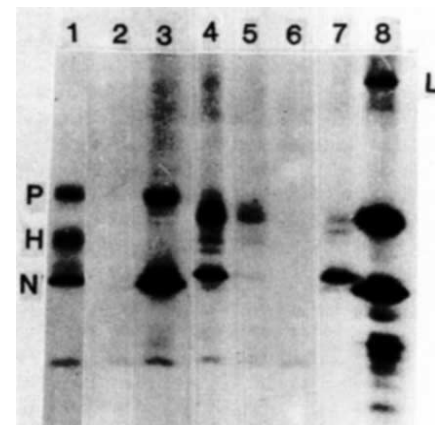
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SIR—Recently we described the pathology of seriously ill and dead seals with a distemper-like illness and the isolation of a morbillivirus from kidney cells of one of these animals¹. The isolate has been characterized further and named tentatively as phocine distemper virus (PDV).

The relationship between PDV, canine distemper virus (CDV) and measles virus (MV) was assessed by examining the ability of hyperimmune dog serum, serum from a patient with sub-acute sclerosing panencephalitis (SSPE), and serum from an infected seal to immunoprecipitate PDV proteins from radiolabelled lysates (see figure). The N protein of PDV was found to have an apparently higher relative molecular mass — 65,000 (65K) — than the N proteins of both MV and CDV (60K), but the serological cross-reactivity shown here precludes virus identification by this method. Therefore, a panel of monoclonal antibodies (mAbs) was used.

Vero-cell cultures, infected with the virus isolate and reference strains of MV, CDV and rinderpest (RPV) were examined by indirect immunofluorescence, using a panel of mAbs to CDV, MV and RPV. No immunofluorescent staining was observed against PDV or four different



Immunoprecipitation and SDS-PAGE analysis of radiolabelled CDV antigen precipitated with hyper-immune CDV dog serum (lane 1), pre-immune dog serum (lane 2), or mAb against N protein (lane 3); radiolabelled PDV antigen precipitated with CDV dog hyper-immune serum (lane 4), infected seal serum (lane 5) SSPE serum (lane 7); mock-infected Vero cell antigen precipitated with seal serum (lane 6) and MV antigen precipitated with SSPE serum (lane 8). The positions of the N, P and H proteins are indicated.

strains of CDV with any of the eight MV mAbs (one anti-N, three anti-M, two anti-H and two anti-F), raised against the LEC-KI strain of MV. All of the MV mAbs reacted with the Human 2 strain of MV. Twelve of the 17 CDV mAbs (raised against the Convac stain of CDV) reacted with PDV whereas staining occurred with all the CDV mAbs against four CDV strains tested (see table). These included two virulent isolates, Snyder-Hill and

Reactivity of mAbs with CDV, MV, RPV, PPRV and PDV

Anti-CDV mAbs	MV (Hu2)	CDV strains ONST	17	RO	SH	PDV	Anti-CDV mAbs	MV (Hu2)	CDV strains ONST	17	RO	SH	PDV
N1 (3.721)	—	+	+	+	+	—	F1 (3.584)	—	+	+	+	+	+
N2 (3.991)	—	+	+	+	+	+	F2 (3.633)	—	+	+	+	+	+
N3 (3.805)	—	+	+	+	+	+	F3 (5.148)	—	+	+	+	+	+
P1 (3.568)	—	+	+	+	+	+	F4 (3.551)	—	+	+	+	+	+
P2 (3.698)	—	+	+	+	+	+	H1 (3.734)	—	+	+	+	+	+
P3 (3.780)	—	+	+	+	+	+	H2 (3.755)	—	+	+	+	+	—
P4 (3.630)	—	+	+	+	+	—	H3 (4.043)	—	+	+	+	+	—
P5 (3.768)	—	+	+	+	+	+	H4 (2.267)	—	+	+	+	+	+
P6 (4.088)	—	+	+	+	+	+							

Anti-RPV mAbs	RPV (RBOK)	PPRV strains	MV (EDM)	CDV (ONST)	PDV
N1	+	—	—	—	—
N2	+	—	+	+	+
N3	+	+	+	+	+
N4	+	+	+	+	+
N5	+	+	+	+w	+w
N6	+	+	—	—	—
F	+	NT	+	+	—
X	+	NT	—	+w	+

Virus strains. MV: Human 2 (Hu2); Edmonston (EDM). RPV: rinderpest bovine Old Kabete (RBOK); CDV: Onderstepoort (ONST); Cornell A75-17 (17); Rockborn (RO) Snyder-Hill (SH). Grading of immunofluorescent staining: +, distinct; +w, weak; —, none. NT, not tested. mAbs H1, H2 and H3 recognize different sites. * Site recognition unknown.

Cornell A75-17 (supplied by M. J. G. Appel, Cornell University), the latter having undergone only two passages in tissue culture. None of the 17 mAbs against CDV proteins reacted with the Human 2 strain of MV. The reactions of 14 of these CDV mAbs against eight other strains of MV and four different strains of RPV have been described previously². mAb F2 reacted with all the MV and RPV strains, mAb N3 with four RPV strains and weakly with six of the eight MV strains and mAb P2 with two RPV strains and one MV strain. All the other mAbs did not react with any MV or RPV strains. Therefore on the basis of their results, PDV was found to be more closely related to CDV than to either MV or RPV. However PDV shows antigenic differences to the four strains of CDV tested in this study as well as the Convac strain, used to prepare the mAbs. As all five strains of CDV (including two virulent isolates) have a high degree of antigenic conservation at the particular epitopes examined the variation exhibited by PDV may indicate that it should be classified as a distinct virus.

Eight mAbs, raised against the rinderpest bovine Old Kabete (RBOK) strain of RPV, were examined for their reaction with the Onderstepoort strain of CDV, and the Edmonston strain of MV and PDV (see table). Six antibodies have been initially characterized as being directed against the N protein and one against the F protein of RBOK with the designation of the eighth mAb (X) unknown. A generalized reaction of the N protein mAbs with a number of different strains of peste des petits ruminants (PPRV) is also indicated. Although a spectrum of cross-reactivity was found with RPV and the other morbilliviruses, differences between the cross-

reactivity of PDV with RPV and the cross-reactivity of CDV with RPV were demonstrated by both the F mAb and the uncharacterized mAb.

These results suggest that the seal-derived morbillivirus is more closely related to CDV antigenically than to any of the other morbilliviruses, but it has a nucleocapsid protein more similar in size to that of some rinderpest strains³. The virus also seems to exhibit unique antigenic epitopes on the N, P and H proteins that distinguish it from both CDV and RPV. To our knowledge, no natural isolates of CDV have been previously made from animals other than dogs even though the

virus is known to infect a wide range of terrestrial carnivores⁴. So we can only compare the seal virus directly with dog isolates. But we suggest that the degree of variation from standard strains of CDV characterizes the virus as a new member of the morbillivirus group rather than as a new strain of CDV. Cloning and sequencing studies of PDV should provide insight into the derivation and classification of the virus, which seems to have been only recently introduced into the seal population⁵.

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Sperm competition in grey whales

SIR—Your legend for the drawing of grey whales taken from Cyall Watson's book *Whales of the World*, perpetuates the myth that a 'helper' male is necessary for successful mating in this species. The legend states that: "Only one male is involved in the actual mating; the other takes an upright position on the far side of the female, acting as a prop or wedge". This description of grey whale mating behaviour, paraphrased from Watson's book, can be traced to Samaras².

All accounts of mating behaviour in grey whales note that more than one male is involved. Although Samaras believed that the most common group consisted of two males and a female, mating groups of up to 18 animals have been observed³. Mating groups may last for up to two hours but change composition as individual males leave or join⁴. These groups probably consist of a single oestrous female and numerous males. Swartz's detailed observations have shown that

mating within these groups is not confined to a single male: the female "repeatedly copulates with more than one male during the same mating bout"^{3,5}. Samaras interpreted the trios of two males and a female sometimes seen in grey whales as a mating couple and an additional 'helper whale' but it is now believed that this is a misinterpretation and that multiple males are copulating with the female and competing through sperm competition in mating groups of all sizes^{3,5}. We have pointed out that the relatively large testes, long penises, copulations of one female with several or many males, and the relatively unaggressive male-male interactions in right, bowhead and grey whales are characteristic of polygynous species where males compete by sperm competition rather than by aggressive interactions⁵.

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These features are found in species throughout the animal kingdom, ranging from insects to primates, where males compete primarily by sperm competition^{5,6}.

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Submarine hot springs and the origin of life

SIR—Miller and Bada¹ argue against the idea² that hydrothermal vents at ocean-ridge crests would have provided suitable environments for life to originate. To their arguments one can add the lack of long-term stable microenvironments that would presumably have been needed for "biochemically inept"³ early replicators⁴ — of whatever chemistry⁵. But similar, less extreme environments are known^{3,6} and could have provided suitable sources of chemical energy and nutrients as well as stable 'culture chambers'.

We have reported^{6,7} fossil hot spring structures formed below 150 °C at the vent sites of 360-million-year-old hydrothermal systems driven, not by magma, but merely by heat stored in the Earth's crust⁸. These fossil vents consist of pyrite (FeS₂) tubes between 0.1 and 10 mm in diameter⁶ associated with hemispherical botryoids also made of pyrite.

We have reproduced similar morphologies by passing ferrous and ferric chloride solution through a 0.5-mm aperture into a sodium sulphide solution at room temperature. Precipitated membranes in the form of narrow iron sulphide tubes and globules were the immediate result⁹. These crystallized to pyrite within 6 months.

Metallurgical separation of metallic sulphides in the froth flotation process exploits the affinity between sulphides and oils¹⁰; pyrite is particularly hydrophobic. Thus organic matter carried in hydrothermal solution might have adhered to, and collected in, porous iron sulphide traps. Within such locally hydrophobic environments it becomes possible to see how condensation reactions such as the polymerization of amino acids might have been brought about. It is known that amino acids can be condensed on dehydrated clay surfaces¹¹. As for the original source of organic molecules, the discovery of abiogenic glycine in Red Sea hydrothermal brine pools¹² illustrates the organic-synthetic competence of mild hydrothermal environments.

It is not known whether conditions 4 × 10⁹ years ago were conducive to the formation of pyrite chimneys, although Lake¹³ has argued for the extreme antiquity of sulphur-metabolizing thermo-

philic organisms. We conclude that the arguments for the origin of life being associated with submarine hot springs remain strong enough to encourage further exploration and experimentation.

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Equable climate in the early Archaean

SIR—The impact of 20–35% lower solar luminosity on the climate of the early Earth has been discussed for almost thirty years. There is no geological evidence for early, near-global, glacial conditions¹ and climate models indicate that defrosting an (almost) completely glaciated planet requires much greater increases in solar luminosity than have occurred. Solutions to the paradox of enhanced early surface temperature despite reduced solar luminosity are radiative: either more of the (reduced) incident solar radiation must be absorbed or more of the emitted infrared radiation must be trapped. Solutions of the latter type are based on arguments that one or more greenhouse gases existed in much larger amounts in the early Archaean atmosphere than in the present one. The former suggestion that the planetary albedo was lower has, until recently, been impossible to examine because in the early Archaean, the Earth was almost certainly dominated by a near-global ocean².

We have integrated the standard coarse resolution Bryan–Cox–Semtner global ocean model¹² for 12 years on a Cyber 205. The only land (Fig. 1) comprises a peninsula and an island, consistent with the estimated timing of continental differentiation³. After 12 years the upper ocean layers had reached equilibrium.

This annual-mean forcing spin-up integration used the Haney⁴ surface boundary condition (newtonian cooling). The annually integrated ocean climate (Fig. 1) exhibits a strong equatorial jet, gyres near the peninsula, and sea surface temperatures of ~289 K at the equator with a less steep latitudinal temperature gradient than that for the present day.

The total heat transport towards the poles in the simulation differs considerably from simulated present-day values (Fig. 2), calculated with the same global ocean model (but for present-day land configuration)⁵. Poleward transport has maxima at about 60° and 20° in both cases but there are differences in magnitude: the Archaean total (largely diffusive) transport has maximum values of ~0.5 × 10¹⁵ W, compared with the modern maximum diffusive transport of ~1.4 × 10¹⁵ W (Northern hemisphere) and ~5.0 × 10¹⁵ W (Southern hemisphere). It is this much reduced poleward transport of heat that is partially responsible for oceanic equatorial temperatures not too different from today's. Indeed a simple atmospheric energy balance climate model predicts equatorial temperatures for the Archaean that are well above freezing, if the meridional transport term is reduced from the present-day value in the ratio suggested by Fig. 2.

We have subsequently performed a more complete simulation by replacing the Haney condition with a full time-dependent surface energy budget and a simple ice model. The immediate response in the Archaean simulation is a decrease in sea surface temperature of ~1.5 K and the winter–summer temperature difference over an annual cycle is ~0.8 K near the equator and 0.5 K in mid-latitudes. But it is important to recognize that neither the 12-year annually forced integration nor the annual-cycle diurnally forced integration are equilibrium simulations; equilibration requires very much longer integration times. An extended box diffusion model, requiring ~1/10,000 of the computer resources needed for the ocean general circulation model (OGCM), with three latitude bands per hemisphere gives relatively good agreement with the OGCM both temporally and at all latitudes; for example, we obtain low-latitude January temperatures of 288.0 K for the box model at 0°–30°, compared with 286.4 K for the OGCM at 15°S, and mid-latitude July temperatures of 278.5 K for the box model of 30°–60° as opposed to 279.5 K for the OGCM at 40°S.

We have considered a single aspect of

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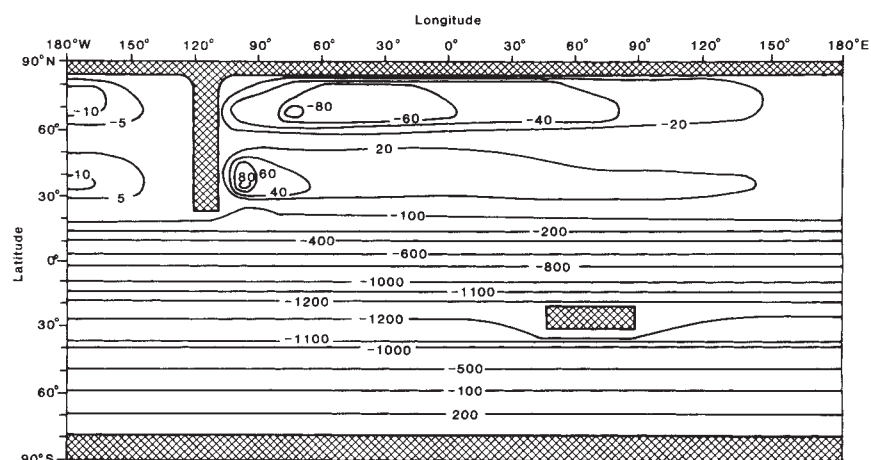


Fig. 1 Streamlines for the 12-year integration of the Archaean global climate model (in units of Sverdrups). The hypothesized land configuration is also shown (cross-hatched); we assume a peninsula (Northern hemisphere) and an island (Southern hemisphere) only. The small polar land areas are required by the OGCM to avoid numerical singularities.

one of the two radiative solutions to the paradox of equable early Archaean temperatures. Once formed, a near-global ocean does not freeze, despite incident solar radiation of only 70% of the present-day intensity. On the contrary, equatorial temperatures lie in the range 13–16 °C with ice extending to ~58° in the mean annual case. Thus both types of radiative solutions to the paradox are viable, and

indeed the geophysical evidence points to both a near-global ocean and greatly enhanced levels of atmospheric CO₂.

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Loss of information in genetic distances

SIR—We certainly cannot dispense with DNA sequences when reconstructing evolutionary trees. Diamond¹ is correct in identifying 'convergence' as one of the important features for tree reconstruction—the tree should not change as more data are collected. But his discussion of the merits of DNA sequences versus DNA/DNA hybridization omits at least one important factor.

The omission is the fact that information is lost in the conversion of DNA sequences to a distance matrix. This loss occurs whether distance matrices are derived from known sequences, or by DNA/DNA hybridization. This conversion is not invertible.

Part of the problem has been discussed previously² when calculating the compression ratio (the ratio of the average number of distinct sets of sequence data, to the number of similarity matrices). So with nine taxa and 20 characters (each of which would have four states), there are, on average, at least 10^{14.9} distinct sets of sequences for each similarity matrix².

This figure is only a lower bound on the information loss because matrices that fail to meet the triangle inequality have not

Examples of average information loss ($\log_{10} \Gamma(n, c)$)

c	$\log_{10} \Gamma(9, c)$	c	$\log_{10} \Gamma(9, c)$
12	3.3	100	178.0
20	18.5	500	798.9
50	79.9	1,000	1,390.4

For example, with $c=20$ there are, on average, at least 10^{18.5} distinct sets of sequences for each distance matrix. The information lost in converting to distances cannot be recovered.

been excluded. It is difficult to calculate the exact proportion that fail the triangle inequality but a lower bound on the loss is easily calculated. For $n=3$ taxa the proportion of similarity matrices that fail the triangle inequality tends to one half. For c characters it is $(3 \times C_3/c^3)$. For larger n , a much greater proportion fail. The result for three taxa gives a lower bound for larger n by using the maximum number of triangles (with independent edges) in a complete graph on n points (a Steiner triple system).

Combining the calculations from the previous paragraphs gives a lower bound on the information loss. Let $\Omega(n, c)$ be the set of essentially distinct sequence spaces on n taxa, four colours (character states) and length c . ('Essentially distinct' means that two sets of data which differ only by a permutation of the sites or by a permutation of character states across sites, are considered identical.)

All matrices derived from sequence data will meet the restrictions $0 \leq d_{ij} = d_{ji} \leq c$ and the triangle inequality $d_{ij} \leq d_{ik} + d_{jk}$ for all taxa, i, j, k , where d is the 'distance' between two sequences. Let $D(n, c)$ denote the set of $n \times n$ matrices satisfying these conditions.

Let $\Gamma(n, c) = |\Omega(n, c)|/|D(n, c)|$. We define this ratio to be the average information loss. In Table 1 we calculate some values for $\log_{10} \Gamma(n, c)$ for different lengths c on four character states for $n=9$ taxa. Examples of the effect of this information loss have been given before².

The reconstruction of evolutionary trees need not be such a controversial field. There are times when distance methods, accompanied by an appropriate analysis³, can give a reliable result. Cladists should accept these cases. That method, however, has not given a clear result with the difficult case of resolving the human, chimpanzee, gorilla trichotomy³. In such cases we need more information than is contained in the distance matrix. DNA sequences will be necessary for the hard problems.

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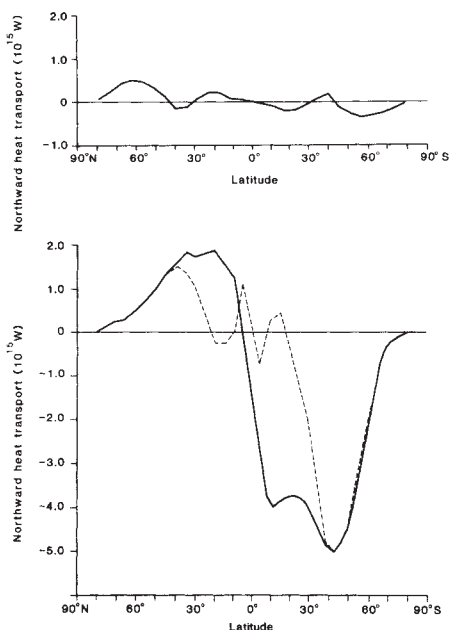


Fig. 2 The Archaean total northward heat transport simulated by the Bryan-Cox-Semtner³ model (upper) can be compared with present-day values calculated by Meehl *et al.*⁵ with the same ocean general circulation model (lower). In the lower figure, the solid line represents total transport and the dashed line indicates diffusive transport. (The comparison should probably be made using the diffusive transport appropriate to the modern ocean, because the total transport is influenced strongly by the intense transport of the land-locked boundary currents, which cannot be present in the Archaean ocean as postulated.)

1. Diamond, J.M. *Nature* **332**, 685–686 (1988).

2. Penny, D.J. *theor. Biol.* **96**, 129–142 (1982).

3. Felsenstein, J.J. *molec. Evol.* **26**, 123–131 (1987).

What makes them tick?

Arun V. Holden

From Clocks to Chaos: The Rhythms of Life. By Leon Glass and Michael C. Mackey. Princeton University Press: 1988. Pp. 248. Hbk \$45; pbk \$13.95.

ONE OF the striking features of living systems is their cyclic nature, the processes from birth to death repeating through generations, annual cycles in populations or in growth of an individual, and through monthly and circadian rhythms. On a shorter timescale there are the periodicities of experimental physiology such as the repetitive discharge of neurons, the heart beat and the slower rhythms of breathing. In spite of the pervasiveness of periodicity biologists still look for steady states — periodic processes are replaced by their mean rates, and the failure to find steady population densities is blamed on the vagaries of the weather.

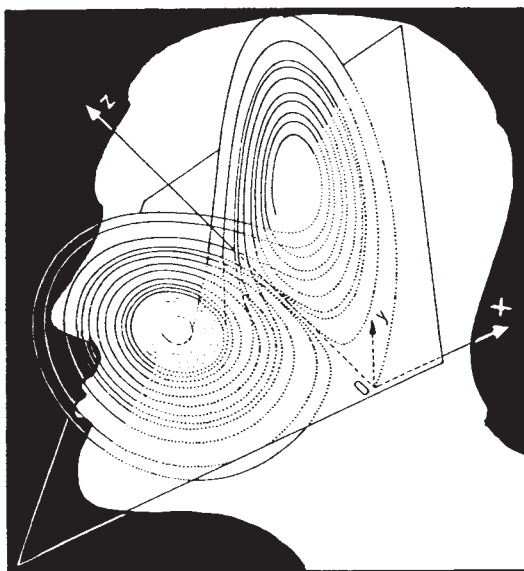
Such naive belief in equilibrium behaviour follows from the practical necessity of usually being able to record only one variable: if a single variable is evolving with time, it must approach some equilibrium value. Although only a single variable may be recorded, however, the processes determining the behaviour often depend on several of them. With the ubiquitous non-linearities of living systems two variables are enough to allow periodicity, and so it is not at all surprising that many biological processes are periodic.

From Clocks to Chaos is the first textbook of general physiology to start with this obvious premise, and to deal with examples using the mathematical methods of nonlinear dynamics. This approach could be a problem for readers from the physiological and clinical sciences, whose training is often deficient in mathematics, but the book is well planned, the mathematics being introduced gently and in contexts that will be familiar to these readers. It also includes an excellent mathematical appendix for those who abandoned mathematics at high school, together with carefully chosen problems that have been field-tested on medical undergraduates.

The authors work at a medical school and share the bizarre humour of medical students; for instance the example given for hard excitation (where a stable equilibrium and a stable periodic state co-exist, as just below a sub-critical Hopf bifurcation) is the sudden onset of bursting activity in pelvic floor musculature following orgasm. Other more

conventional examples come from all areas of membrane, cellular and systems physiology, and the generation, maintenance and responses to perturbation of oscillations are illustrated.

Part of the reluctance of experimental and clinical physiologists to accept the methodologies of nonlinear dynamics is that biological oscillations are not periodic: there is some irregularity, and so



an empirical classification into types A, B or C (or whatever) is easier and more sensible than talking about strictly periodic limit cycles. This irregularity can be produced by stochastic processes, which are described by probabilistic, non-deterministic equations, or can in fact result from deterministic nonlinear dynamics as chaos.

It is well known that stochastic processes, or noise, occur in physiology: examples include the time sequence of miniature end-plate potentials or of the intervals between nerve impulses. This subject has not generated great excitement and has been left to mathematically orientated biophysicists. However, ever since the appearance of Robert May's review article in 1976 (*Nature* 261, 459–467) there has been an enthusiastic, almost evangelical search for chaotic behaviour in biological systems and investigation of its possible significance. Attitudes to the subject range from those who believe that chaos is of little importance to those who think that chaotic dynamics is the driving force for evolution, adaptive behaviour

and even consciousness. The drift from analysis to speculation mirrors the increase in entropy illustrated by the statistical physicist's ditty that takes us from Shakespeare to Sinatra, via Sartre:

To be or not to be,

To be is to do

To do is to be

Doobie Doobie Doobie Doo;

from a detailed analysis of period doubling bifurcation sequences in one-dimensional return maps one soon ends up seeing period doublings and chaos everywhere.

Glass and Mackey do not follow this fuzzy-minded approach, but instead present a solid, methodical account of the dynamical behaviour of biological systems: chaos is an important but small

part of the behaviour. Their notion of a *dynamical disease*, where pathology is associated with abnormal dynamics, is not a superficial link between chaotic dynamics and pathophysiology; in some systems chaos appears to be normal, and periodicity pathological.

Although there are well-developed quantitative tests for identifying chaotic behaviour which have been applied to a variety of biological problems, they are sophisticated and not readily accessible to biological readers. The section in the book which deals with distinguishing noise from chaos is invaluable in allowing biologists to read, and assess, the chaotic literature on biological chaos. Spatial, as well as temporal, periodicities are considered.

Here an important clinical area of interest is the role of circulating spiral waves in two dimensions, as a mechanism in ventricular fibrillation. Because experimental measurements of spiral and scroll waves are only just beginning, some of the problems raised may be answered in the next decade.

So far I have considered the viewpoint of biologists, who have a real need for the methods expounded in this volume. It will also be invaluable to those applied mathematicians whose interests sometimes veer into physiology, as it provides a survey of problems in physiology in a language that they will readily appreciate. When *From Clocks to Chaos* is read by applied mathematicians, physiological research will benefit; when it becomes widely used in medical physiology courses, the shape of medicine will radically change from its present emphasis on structure and local mechanism to a more global appreciation of behaviour in an interacting, complicated dynamical system. □

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A parasite exposed

Margaret Perkins

Malaria Immunology. Edited by P. Perlmann and H. Wigzell. Karger: 1988. Pp. 372. SwFr.262, £119.10, \$174.75.

MALARIA remains one of the most devastating of human diseases. In the past decade, researchers have rapidly (and anxiously) applied the technologies of molecular biology and immunology to identify plasmodial antigens and test their immunological importance. *Malaria Immunology*, a collection of 12 reviews which have been edited expertly by Peter Perlmann and Hans Wigzell, documents the resulting, prodigious growth in our knowledge and the steady progress towards the ultimate goal — the development of a vaccine for human malaria. The book will be of use not only to researchers inside the field but to those generally interested in immunity to infectious diseases.

Mosquitoes transmit malaria by injection of sporozoites into the blood, and thus it is appropriate that the first two chapters are concerned with this stage. The coat of the sporozoite is composed of a single antigen, the CS (circumsporozoite) protein first identified and characterized by Ruth Nussenzweig and colleagues. Given its pre-eminence as a vaccine candidate, the CS protein has been the most intensely studied of all malarial antigens. Both B- and T-cell epitopes of the antigen have been identified and their relative importance in the immune response is currently being examined. The complex task of unravelling the immune response to the CS protein is well analysed in Chapters 1 and 11.

In contrast to the relative simplicity of the antigenic make-up of the sporozoite stage, the blood stages of plasmodia have presented us with an overwhelming number of antigens, many of them highly immunogenic. The sheer number of antigens probably increases parasite survival by diluting the immune response to critical antigens. But choosing which of the many antigens or epitopes will be successful immunogens for vaccination is still a matter of debate.

The properties of the blood-stage antigens are discussed in Chapters 3–7; although there is some overlap in material between them, most aspects are well covered. Anders and colleagues provide a valuable summary of the structure of many of the antigens that have been sequenced, the most dramatic feature of which is the presence of highly repetitive sequences (as many as ten), often constituting more than 80 per cent of the protein. Although there are no real clues yet as to what advantage these antigens confer on

the parasite, there is speculation that repetitive structures may play a role in immune evasion. The discussion of receptor-mediated erythrocyte invasion by the parasite is confusing on certain points, and perhaps the topic could have been better explained with a few clear diagrams. The contribution on immunity to the sexual stages gives a well-balanced picture of the potential advantages and problems of developing an appropriate vaccine.

From the results of recent animal and human malaria vaccine trials, it has become apparent that T-cell epitopes of antigens will also have to be included in future trials. Chapters 9 and 10 of the book give a comprehensive survey of the current literature on the role of T-cell immunity, an area that is increasingly gaining attention. The success of the malarial parasite is attributed in large part to the novel strategies it has devised to avoid immune destruction in the host. One aspect of this that receives little discussion is the question of why individuals in endemic areas are repeatedly infected with the disease in the face of a highly efficient production of anti-plasmodial antibodies.

In their preface Perlmann and Wigzell caution us that, despite the extraordinary progress in malaria research in the past decade, a vaccine is not just "around the corner". But since their book went to press, there have been breakthroughs. Manuel Patarroyo and colleagues (*Nature* 332, 158; 1988) have successfully immunized humans against *Plasmodium falciparum* by using a synthetic polymer composed of peptides of several different blood-stage antigens. Although *Malaria Immunology* demonstrates that we have a lot more to learn about the immune response to this complex and entrenched parasite, these developments certainly give us cause for optimism. □

Margaret Perkins is in the Department of Biochemical Parasitology, The Rockefeller University, 1230 York Avenue, New York, New York 10021, USA.

New in paperback

- *Eat Your Heart Out* by J. Le Fanu. Publisher is Macmillan, London, price is £5.95. For review see *Nature* 330, 284 (1987).
- *QED: The Strange Theory of Light and Matter* by R.P. Feynman. Publisher is Princeton University Press, price is \$8.95. To be published in Britain by Penguin. For review see *Nature* 320, 661 (1986).
- *Fabry-Perot Interferometers* by G. Hernandez. Publisher is Cambridge University Press, price is £15, \$29.95.
- *Modeling Nature: Episodes in the History of Population Ecology* by S.E. Kingsland. Publisher is Chicago University Press, price is \$11.95, £9.50. For review see *Nature* 322, 603 (1986).
- *Unfinished Synthesis* by Niles Eldredge. Publisher is Oxford University Press, price is £11.95, \$14.95. For review see *Nature* 320, 649 (1986).

Origins in heat and dust

David W. Hughes

The Solar System: Chemistry as a Key to its Origin. Edited by S.K. Runcorn, G. Turner and M.M. Woolfson. *The Royal Society, London: 1988. Pp. 251. £50, \$90.50.*

THE Solar System that we observe today is about 4,570,000,000 years old and the mass is now a mere 1/750 that of the central Sun. Four things are clear about it. First, most of this mass is confined to a thin disk-like region that has a radius 30 times greater than the radius of the orbit of Earth. Secondly, the size of the planetary orbits has changed little with time. Thirdly, the flattened, doughnut-shaped nebula cloud of gas and dust was initially hundreds of times more massive than the planets that formed from it. And finally, chemistry played a large part during the condensation and accretion processes that resulted in the planets.

Most cosmogonists (those who study planetary formation processes) believe that the nebula was produced by the condensation of interstellar material, material similar to that from which the Sun was produced. This material can be crudely divided by mass into 1 part of metal and rock, 2.2 parts of ices (H_2O , CO_2 , CH_4 and NH_3) and 200 parts of gas (H and He). In the hot inner regions of the nebula only the rock and metal could condense. Moving out beyond the asteroid belt, ices and gases condensed onto the rocky/metal nucleation centres and giant planets like Jupiter were eventually formed.

The Discussion Meeting at the Royal Society, held on 15 and 16 July 1987, was timely and brought together many of the world's foremost astrochemists. The papers presented at that meeting have been brought together to form this impressive book.

The timeliness was because a number of clues, together giving insight into the chemistry of the pre-planetary nebula and the present Solar System, had become available over the preceding years. For example, the Moon rock that was returned to Earth in the early 1970s had been carefully analysed; here we have an extra-terrestrial sample of material formed at about 1 astronomical unit from the Sun. In 1969, a primitive carbonaceous chondrite meteorite fell to Earth at Allende in Mexico, providing a sample from the asteroid belt. And 1986 saw a spacecraft fly-by of a mass of primitive outer-Solar-System dust and snow in the form of the nucleus of Halley's Comet. The chemical evidence, and particularly the abundances of the

elements and isotopes, obtained from these sources were taken as the starting point for the meeting.

The first section of *The Solar System* concentrates on the nucleosynthetic processes responsible for the chemical composition of interstellar gas clouds and the Sun, while the next two deal largely with meteorites; the fact that these can be picked up, taken into laboratories and analysed in detail means that much is known. The book then returns to the more uncharted reaches of the subject — the chemistry of comets, the atmospheres of the Jovian planets and the environments of the planets at the time when the first traces of life started to evolve.

Altogether, this is an enormous topic, and one crying out for more data. It is also clear that there are many problems that will be hard to overcome. We can foresee, in the near future, space-borne instrumentation orbiting and landing on other

planets and satellites. But we cannot go back in time to those early days of dust, gas and planetesimals, when accretion and fragmentation were vying with each other and when radioactive, solar and kinetic energy heat sources were more dominant. It will be many years before we stop being embarrassed by the fact that we are still mystified by many of the processes that helped produce our planet and its companions.

The value of the book lies not only in the plethora of excellent, authoritative contributions summarizing the state of chemical cosmogony, but also in the well-documented discussion sections. Here we can savour in full the scientific excitement to be had in the subject, and can appreciate the host of new ideas that are waiting to be tested. □

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Believe it or not

Paul Davies

A Physicist's Guide to Skepticism. By Milton A. Rothman. *Prometheus: 1988. Pp. 247. \$19.95, £14.95.*

ALTHOUGH we live in a so-called scientific age, many people do not share the scientist's view of reality. Opinion polls suggest that belief in psychic phenomena, unidentified flying objects, astrology and other bizarre ideas is widespread. The curious thing about many of these beliefs is that although they fail to meet the exacting standards of scientific rigour they are frequently discussed in a superficially scientific manner. Thus arises the concept of pseudoscience.

The pseudoscientist makes much use of scientific terminology. There is talk of fields, black holes, energy flows, vibrations and quantum waves. To the layman this sort of jargon can make pseudoscientists seem erudite and their claims convincing. As a result there has been an explosion of commercially successful books on a whole range of dubious topics, from time travel to telepathy, couched in the bastardized language of pseudoscience. Indeed, so numerous are these texts that genuine scientific books are often subsumed under 'The Occult' in book stores. As most science popularizers know to their chagrin, a considerable amount of apparent interest in science actually stems from a fascination with pseudoscience. Thus many people want to find out about quantum mechanics not in order to better understand the workings of the atom, but out of a wild belief that it will somehow explain precognition or life after death. In my experience, even casual

conversation about physics with ordinary folk is soon steered away from the real thing and turns to topics such as ghosts or UFOs.

Milton Rothman is not the first scientist to strike back. His book is a brave attempt to tackle the insidious advance of pseudoscience by carefully explaining in non-specialist terms the aims and procedures of genuine science, and comparing the nature of scientific claims with those of the pseudoscientist. This is not an easy task. The question of what makes a scientific claim, such as the link between smoking and cancer, reliable, and the assertion that astrology can foretell the future unreliable, is a subtle one.

Much of the book is devoted to surveying basic physics and explaining such things as the law of conservation of energy and the second law of thermodynamics. There is an instructive study of the history of perpetual motion machines, and why they cannot work, as an illustration of the use of scientific reasoning. There follows a philosophical discussion of the laws of physics, and the deep issue of verification and falsification of hypotheses.

Those readers who expect a systematic put-down of pseudoscientific topics will be disappointed. The author is more concerned to dwell on general methodology and mythology than to debunk specific claims. He is probably right in this. The committed believer will never accept rational argument against his or her pet belief anyway. The best that can be achieved in a book of this sort is that tender readers will acquire a modicum of scepticism to set against the outpourings of pseudoscientific propaganda to which they are regularly subjected. □

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Genes evolving

T. Cavalier-Smith

Four Billion Years: An Essay on the Evolution of Genes and Organisms. By William F. Loomis. *Sinauer*: 1988. Pp. 286. Hbk \$39.95, £29.95; pbk \$22.95, £17.50.*

UNTIL we know how genes make daisies rather than dinosaurs, the present gulf between molecular and organismic evolutionists will remain. Any book on the evolution of genes and organisms is therefore bound to be somewhat disjointed.

Loomis's readable essay is much less coherent than it need be, however. The reason is that the author — like so many biochemists — seems rather ignorant of the structure of organisms; in particular, there is here all too little appreciation of the structural diversity of unicellular organisms, for which we are much closer to understanding the molecular basis of body form than we are for animals and plants, and therefore also their organismic evolution. The book is even weaker on phylogeny, which is scarcely discussed at all — a curious omission for an essay on the main steps in the evolution of life. Too few molecular biologists realize that without an accurate phylogeny one cannot even correctly identify these steps, let alone discuss them intelligently. Nor, without a detailed knowledge of the actual structure, development and life history of the specific types of organism actually involved in a particular step, can we realistically apply what little we yet know of molecular epigenetics to explaining how major new types of organism evolve.

Loomis is also shaky on mechanisms of evolution, failing to appreciate the central role of reproductive competition. This is seen not only in remarks such as "all selection is for survival" and "fertility is seldom a limiting factor", but also in the brevity of his reference to arguments invoking the role of 'selfish' DNA in genomic evolution. Given the neglect of both phylogeny and the idea of intragenomic spreading of DNA sequences, it is not surprising that he unquestioningly accepts the over-popular primordial gene fusion theory (rather than the transposon insertion theory) of the evolutionary origin of introns.

Most surprising, though, is the treatment of eukaryote cell evolution, which goes counter to the recently emerging consensus. Even though Loomis accepts the symbiotic origin of both chloroplasts and mitochondria, he thinks that the eukaryote (host) cell itself evolved from a photosynthetic cyanobacterium. He is unaware of the existence of eukaryotes without mitochondria, and their probable

evolutionary significance, and of the extensive writings of evolutionary protistologists over the past dozen years or so. Equally idiosyncratic is the overriding importance given to rising oxygen levels for everything from the 'loss' of bacterial introns to the extinction of trilobites and dinosaurs, and even the origin of placental mammals, birds, antelopes and woolly mammoths.

The discussion of the molecular evolution of animal form is interesting and sensible as far as pattern formation is concerned. But it is weak in its neglect of the physical basis of morphogenesis, and in its phylogenetic and morphological misconceptions. Loomis, it seems, believes that sponges are a mere colony of flagellates.

Apart from the account of the genetics of pattern formation, the book's main strength lies in the way the author illustrates its pervasive theme of gene divergence from ancestral genes by the use of aligned motifs from actual protein sequences. The conservation of gene sequences and the importance of both gene duplication and the formation of new chimaeric genes (a process I would call gene chimaeration) are made very clear. This, however, is very much a book by a biochemist. It is thin on cell and organismic evolution, abysmally so when it comes to plants, fungi and protozoa, to which little more than a couple of pages are allocated. The discussion of prebiotic and metabolic chemistry is pretty conventional.

Loomis says of his book that "The primary goal is to connect the experimental and conceptual facts into a coherent story rather than to analyse the strengths and weaknesses of each step along the

way". But to whom is this story directed? Not to the 'intelligent layman', for there is too much biochemistry for that. Nor to the professional evolutionist — the author makes no claims to be presenting either new ideas or a critical analysis. The problem with not examining the strengths and weaknesses of the steps along the way is that the inexperienced reader has no way of knowing which parts are firmly based (for example the symbiotic origin of chloroplasts) and which (for example the assumption that the first cells were fermenters rather than photosynthesizers) are based on little more than fashion. This is more a personal travelogue than a traditional work of science: one may learn as much if not more about the author's background, interests and limitations as about the places visited.

I echo Loomis's hope that his book will encourage others to attempt a broad synthesis of molecular and organismic approaches to the history of life on our planet. But let them do their homework and learn more about organisms and phylogeny, more about the actual fossil record, and more about evolutionary mechanisms before putting pen to paper. We need much more than 'thermodynamics and biophysics' to constrain us 'from wild speculation'. Although *Four Billion Years* contains much more molecular detail than Lucretius, in its quasi-linear approach it seems closer to him and to eighteenth-century transformationists in intellectual flavour than to modern post-darwinian evolutionary biology. □

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All a-flutter — a cluster of monarch butterflies overwintering in coastal California. The photograph (by E. S. Ross) is taken from the fourth edition of *Animal Behavior: An Evolutionary Approach* by John Alcock. This edition has been largely rewritten: "the most important change . . . is my attempt to emphasize the tentative, unfinished nature of science and the significance of using multiple working hypotheses". Publisher is Sinauer, price is \$34.95, £24.95 (for the British distributor see note opposite).

* In Britain distributed until now by Blackwell Scientific, but from November 1988 by W.H. Freeman.

Trophic regulation of nerve cell morphology and innervation in the autonomic nervous system

Dale Purves, William D. Snider* & James T. Voyvodic

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A remarkable feature of nerve cells is the complex and variable pattern of their axonal and dendritic branches. Quantitative studies of a simple part of the nervous system in mammals provide evidence that neuronal geometry and innervation are regulated by long-term trophic interactions between neurons and their targets. This trophic linkage may explain how nerve cells adjust their function to the needs of bodies that vary markedly in size and form.

THE autonomic nervous system, which regulates the involuntary functions of the body in mammals and other vertebrates, includes many nerve cells that lie outside the brain and spinal cord. The accessibility of these neurons in autonomic ganglia (see box) has allowed a detailed analysis of the relationship of the morphology of nerve cells to the targets they innervate and the innervation they receive. These studies indicate that both the geometry of autonomic neurons and their innervation are governed by ongoing interactions with the tissues they innervate. The conclusion that the form and innervation of nerve cells are influenced by the body is relevant to understanding both the development and evolution of the nervous system. In both contexts, we suggest that neurons use trophic signals derived from their target tissues continually to adjust their form and innervation to changes in the size and form of the body.

Trophic interactions

Trophic interactions are defined operationally as the long-term interdependent relationships of neurons and the cells they innervate, which may be either non-neural cells or other neurons. Long-term denotes effects that take place over days, weeks or

months; interdependence refers to the deleterious effects observed when one of the elements in the partnership is removed. Trophic interactions are generally thought to involve a specific molecular message that passes between the two cells, in addition to whatever neurotransmitters are released during synaptic transmission. The word 'trophic' is used because it implies a nutritive or maintenance factor (the Greek *trophē* means nourishment).

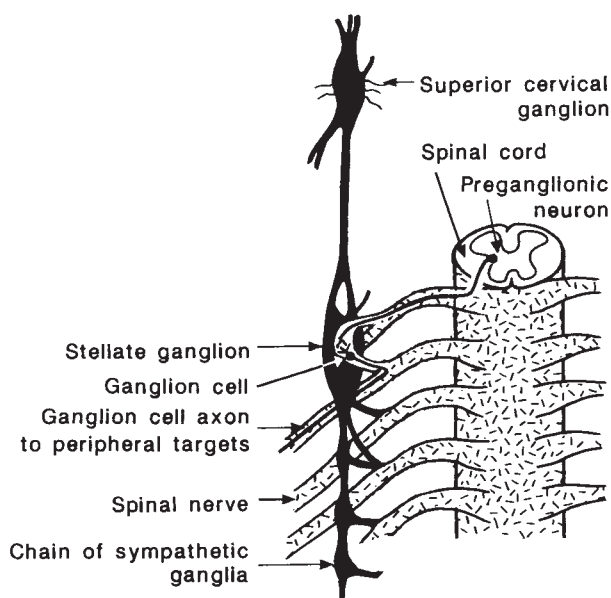
Trophic interactions, so defined, were originally proposed to explain the developmental regulation of neuronal numbers^{1,2}. Here we argue that patterns of nerve cell connections, that is the arrangement of axonal and dendritic arborizations and the synaptic connections they make, are also subject to trophic regulation that continues throughout life.

Neuronal form in ontogeny and phylogeny

It is generally true of mammals that the final number of nerve cells is defined early in ontogeny, often in the embryo³⁻⁵. On the other hand the growth of the body, which occurs by the addition of more cells and the enlargement of pre-existing ones, persists well into the postnatal period in all mammals and for most of life in some species. A relatively fixed number of nerve

The mammalian autonomic nervous system

The autonomic nervous system includes large numbers of neurons outside the brain and spinal cord organized into clusters called ganglia (shown black). The axons of these ganglion cells project through peripheral nerves to targets such as smooth muscles and glands that control the many involuntary functions upon which the homeostasis of mammals depends, for example, heart rate, vascular tone and glandular secretion. The nerve cells in the ganglia are themselves innervated by preganglionic neurons with cell bodies in the spinal cord and brainstem. The autonomic system is usually considered as three broad divisions; sympathetic, parasympathetic and enteric (innervating the gut; not discussed in this article). The sympathetic division (illustrated here) consists of segmentally arranged ganglia that lie along the length of the vertebral column, relatively far from their target organs; the neurons of these ganglia typically synthesize the transmitter noradrenaline. Only the first few thoracic spinal segments are shown here. The ganglia of the parasympathetic division, in contrast, are usually closely associated with their peripheral targets, which they activate by release of the transmitter acetylcholine. They include the submandibular and ciliary ganglia mentioned in the text. The peripheral autonomic system is particularly appealing for investigating trophic relationships because of its accessibility and the relative simplicity of its organization compared to pathways entirely within the central nervous system. Ganglionic neurons can be readily impaled with microelectrodes and their innervation and relation to targets assessed by electrophysiological and anatomical means.



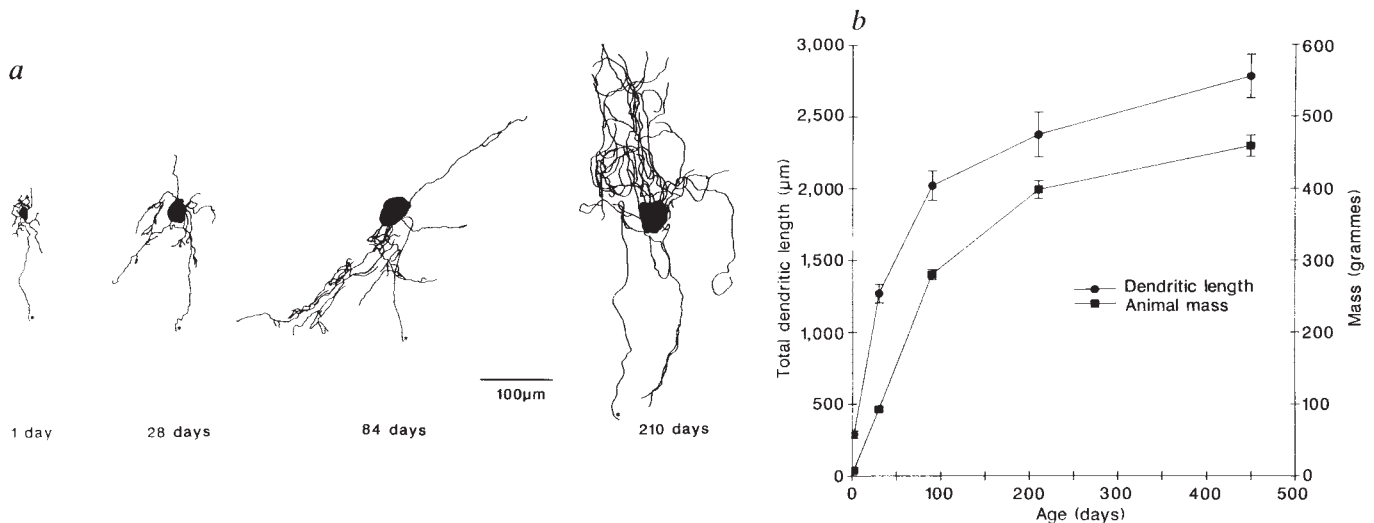


Fig. 1 The length of ganglion cell dendrites and complexity of their branching increases as the body grows, shown here for the rat. *a*, Typical superior cervical ganglion cells at various ages in days from birth to maturity. The neurons were injected intracellularly with the enzyme horseradish peroxidase, treated to produce a visible reaction product and traced using a camera lucida; asterisks indicate the axon of each cell, which extends much further than shown here. *b*, Corresponding graph of the average dendritic length from samples of ~50 neurons measured at various ages. The growth of dendritic branches follows the overall growth of the animal, measured as body mass. Bars indicate standard errors of the mean (after ref. 7).

cells must therefore innervate a body that changes progressively in size and number of constituent cells. This implies that the nervous system remains informed about its changing targets to ensure efficient neural function during a prolonged period of somatic growth.

The relation of neuronal form to the growth of neural targets has been studied explicitly in the mammalian sympathetic system (Fig. 1). In the autonomic nervous system, the axonal and dendritic arborizations of ganglion cells are rudimentary in neonates and increase in length and complexity for as long as the animal, and the neural targets it comprises, continues to grow (Fig. 1)^{6,7}. This suggests that, after the final number of neurons has been established in early life, further adjustment of neuron morphology to growth of the body occurs by the ongoing elaboration of axonal and dendritic branches.

A similar relationship is apparent in phylogeny. Taking a range of mammals from mouse to rabbit, in which there is a 75-fold increase in body size, the number of cells in the superior cervical ganglion (see box) increases only fourfold and the

number of neurons in the spinal cord that drive these ganglion cells increases twofold (Table 1a)⁸; the same disproportion has been found in the sympathetic system of primates^{9,10}. Thus, evolutionary changes that result in large differences in animal size do not involve commensurate changes in the numbers of autonomic neurons. This conclusion may also apply to other types of nerve cells, as it has long been noted that brain weight, which presumably reflects the number of cells in the central nervous system, fails to keep pace with the body weight of progressively larger animals^{11,12}.

As in development, the nervous system seems to cope with these phyletic disproportions between body size and neuronal number by modulating the morphology of nerve cells (Fig. 2). In a small animal like the mouse, the dendritic arborizations of cells in the superior cervical ganglion are simpler, using several criteria of dendritic complexity, than are the homologous cells in a much larger mammal like the rabbit¹³. A similar relationship has been described in parasympathetic ganglia, in which neurons are usually morphologically less complex than sympathetic

Table 1 The innervation of superior cervical ganglion cells in mammals of different sizes

Species	Adult mass of animals (g)	<i>a</i>		<i>b</i>	
		Number of superior cervical ganglion neurons	Number of preganglionic neurons innervating the superior cervical ganglion	Mean number of preganglionic neurons innervating each ganglion cell	Mean number of ganglion cells innervated by each axon
Mouse	25	10,000	700	4.5	64
Hamster	100	17,000	700	7.2	180
Rat	160	26,000	1,000	8.7	237
Guinea pig	400	36,000	1,300	12.3	330
Rabbit	1,600	41,000	1,500	15.5	422
Macaque	4,000	243,000	4,900	ND	ND
Baboon	13,500	397,000	6,500	ND	ND
Chimpanzee	38,000	753,000	7,700	ND	ND
Man	53,000	911,000	8,300	ND	ND

The values have been rounded off⁸⁻¹⁰. The number of ganglion cells innervated by each axon is calculated by multiplying the ratio of ganglion cells to preganglionic cells by the mean number of preganglionic axons innervating each ganglion cell. ND, not determined.

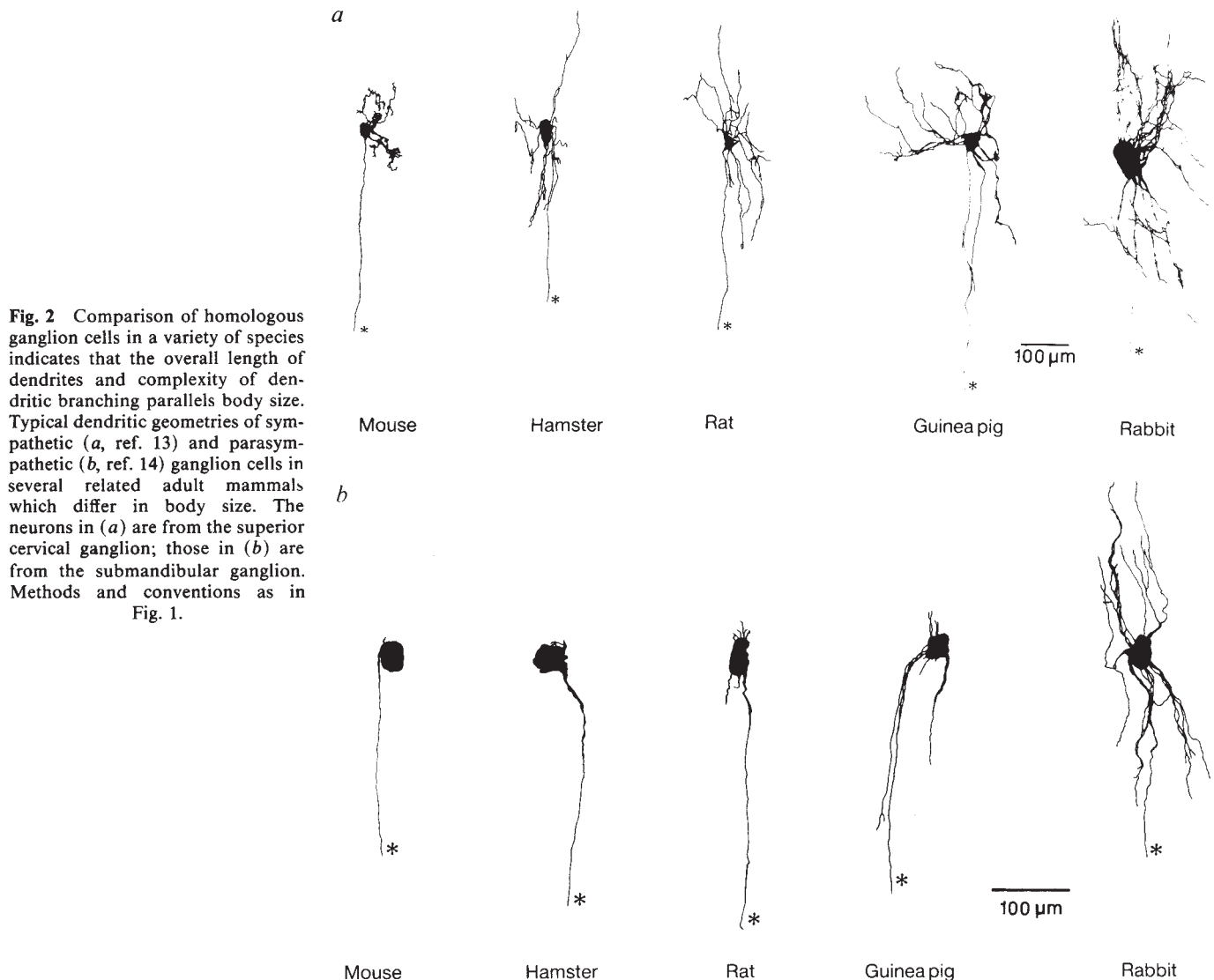


Fig. 2 Comparison of homologous ganglion cells in a variety of species indicates that the overall length of dendrites and complexity of dendritic branching parallels body size. Typical dendritic geometries of sympathetic (*a*, ref. 13) and parasympathetic (*b*, ref. 14) ganglion cells in several related adult mammals which differ in body size. The neurons in (*a*) are from the superior cervical ganglion; those in (*b*) are from the submandibular ganglion. Methods and conventions as in Fig. 1.

ganglion cells¹⁴. In both ontogeny and phylogeny, therefore, a relatively fixed number of autonomic nerve cells seems to adjust to body size by varying the complexity of the branches of individual neurons.

Functional consequences

The geometry of neurons in autonomic ganglia affects the number of inputs (preganglionic axons) that contact each neuron: neurons with more complex dendritic arborizations are innervated by more axons than those with few or no dendrites (ref. 15; Fig. 3). These parallel changes in dendritic geometry and number of inputs have been observed in a variety of ganglia in the same species^{13,14,16-18}. The correlation between the number of innervating axons and dendritic complexity is also evident in comparisons between homologous neurons of different species^{13,14}.

The importance of these systematic differences in neuronal form and innervation is apparent in comparisons among species. The disproportion between neuronal number and increasing body size (Table 1a) implies increased terminal branching of the axons in larger animals, because relatively few neurons are obliged to contact more and/or larger target cells. Axonal branching has been assessed in homologous sympathetic ganglia in a range of species by measuring the average number of ganglion cells contacted by each preganglionic axon. In general,

this value increases systematically across species in relation to animal size⁸ (Table 1b). The degree to which axon terminals of ganglion cells branch among their target cells must also increase progressively in larger animals, although this assumption has never been assessed directly. Increased branching of preganglionic nerve cells in the ganglia and of ganglion cell terminals in peripheral targets means that an action potential in a preganglionic neuron or ganglion cell in a rabbit will activate more target cells than the corresponding action potential in a mouse. Moreover, the larger the number of inputs converging on a ganglion cell, the higher the average rate of its synaptic activity¹⁹. Because each autonomic neuron in a larger animal activates more target cells at a higher rate, a disproportionately small number of autonomic ganglion cells can achieve the same functional effect in a rabbit as in a mouse. Modification of neuronal form thus provides a means by which the autonomic nervous system can adjust aspects of its function to the requirements of different body sizes.

Trophic regulation

The observation that the size of axonal and dendritic arborizations varies according to the size of peripheral targets in both ontogeny and phylogeny (Figs 1 and 2) provides circumstantial evidence that the form of autonomic neurons is regulated by trophic interactions between the neurons and their targets.

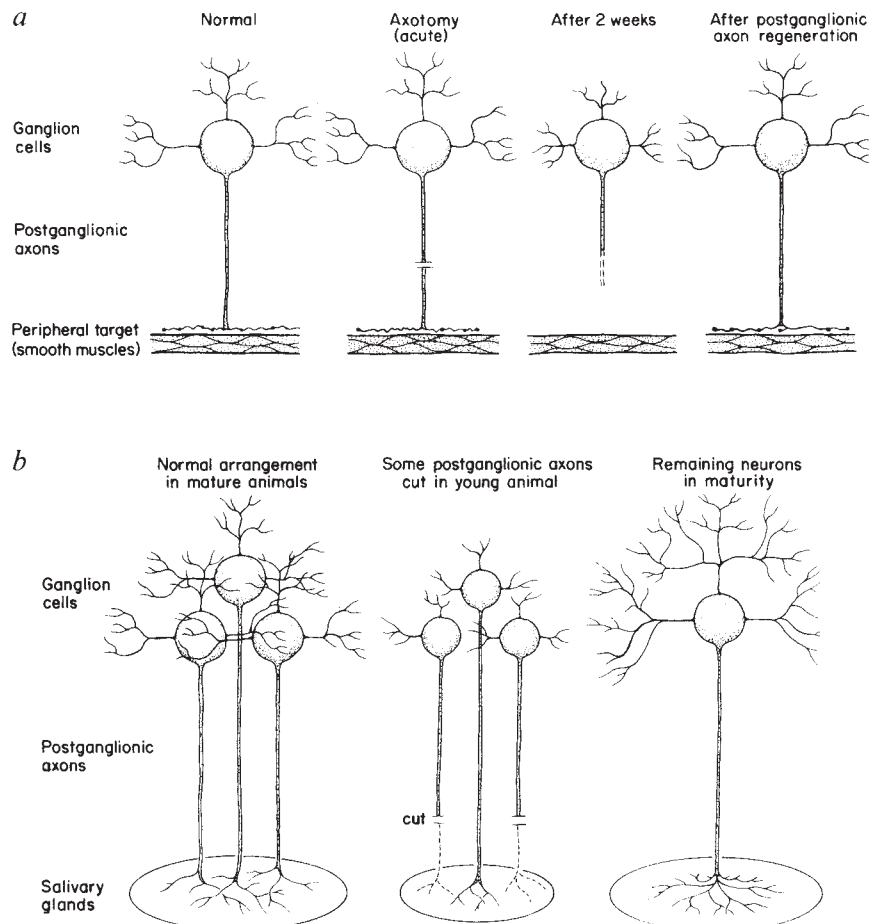
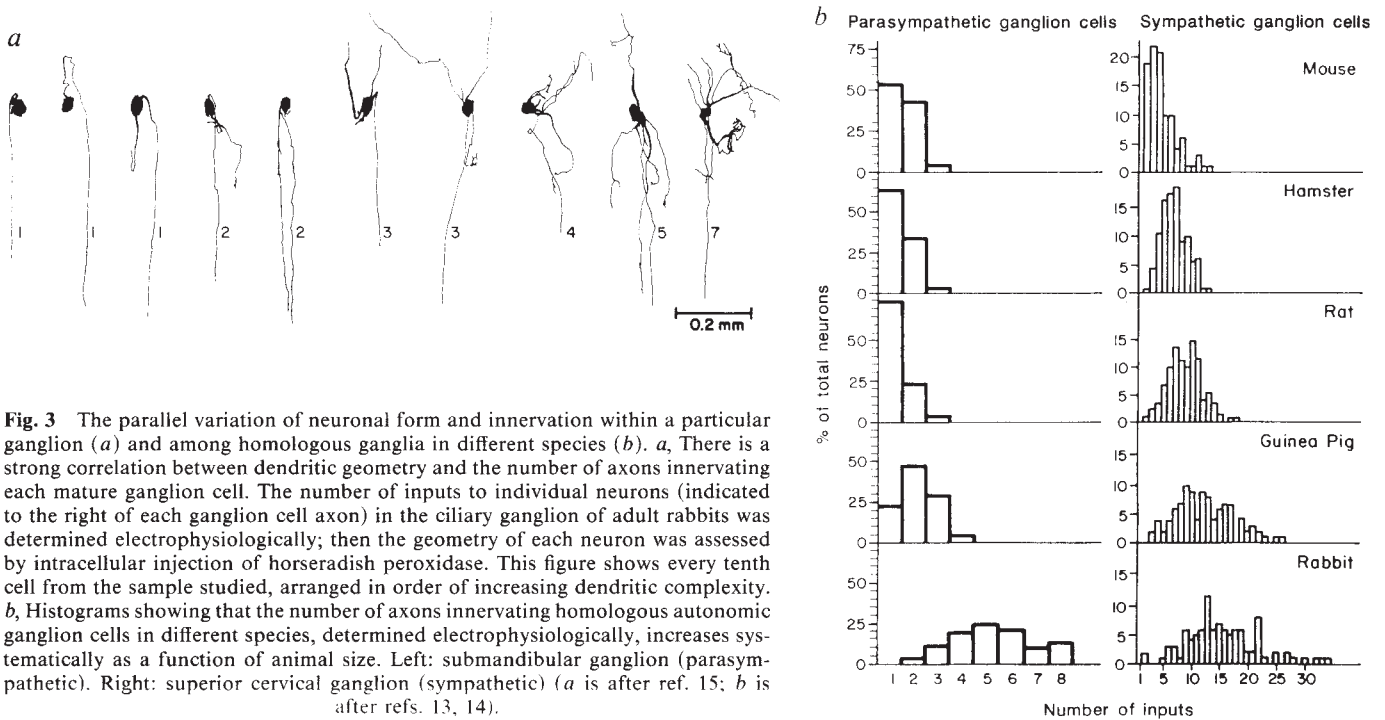
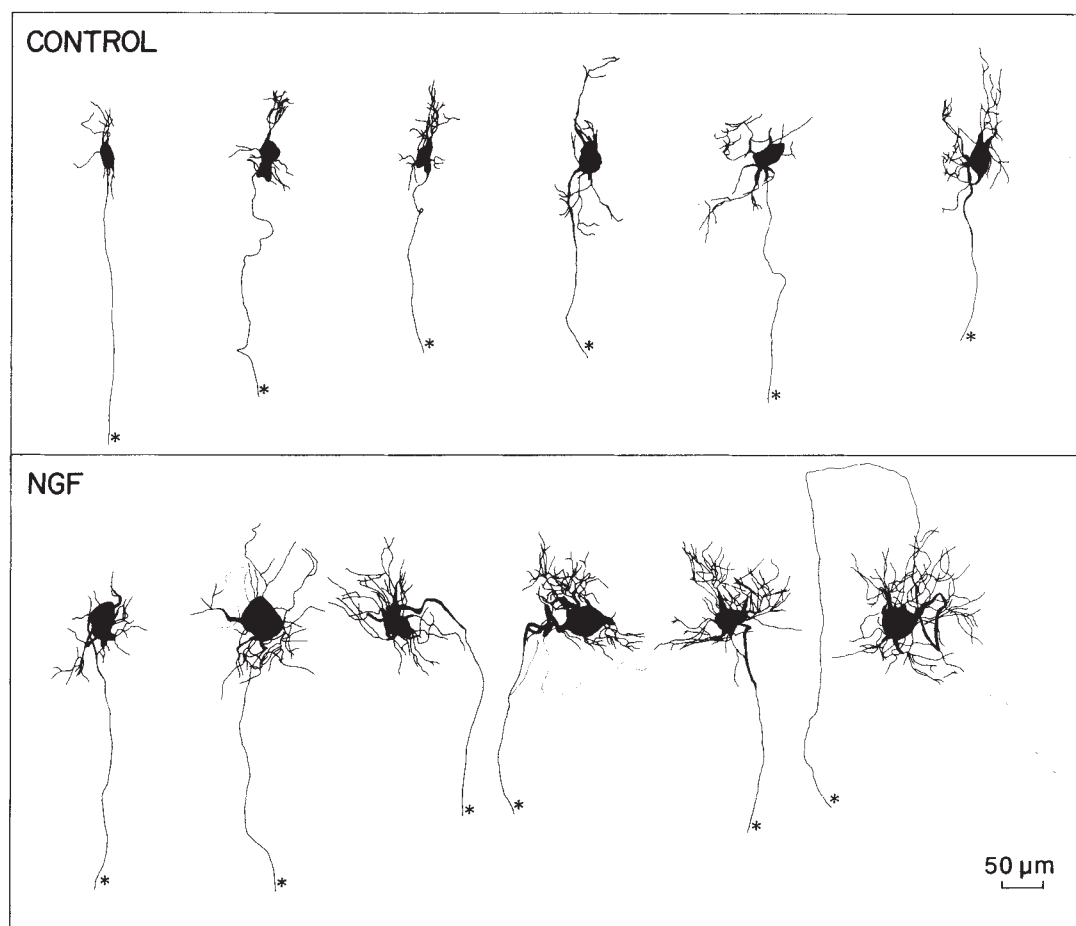


Fig. 5 Developing dendritic arborizations are influenced by nerve growth factor. Daily administration of nerve growth factor to neonatal rats for two weeks results in a marked increase in the elaboration of dendrites of superior cervical ganglion cells compared to neurons in age-matched controls. These and related studies suggest that specific trophic agents such as nerve growth factor are the signal that links neuronal morphology and innervation to the status of peripheral targets³⁹.



Evidence for the hypothesis that the form of autonomic neurons depends on continuous trophic support from their targets comes from studies in which the axons connecting ganglion cells to their peripheral targets have been cut²⁰. After axotomy, the dendritic trees of sympathetic ganglion cells retract for about two weeks and gradually re-expand as peripheral re-innervation proceeds (Fig. 4a). More direct evidence comes from experimental manipulations that change the amount of target tissue innervated by autonomic neurons (Fig. 4b; ref. 21 and unpublished results). When the ratio of target cells to ganglion cells is increased, the dendritic arborizations of the affected neurons become larger and more complex. Conversely, when the ratio is decreased, the dendritic trees are smaller and simpler than normal.

The innervation of ganglion cells also seems to be regulated by trophic interactions. For example, in neonatal rabbits each ciliary ganglion cell is innervated on average by five axons²². As the animal matures, neurons that lack dendrites lose most of their inputs, whereas ganglion cells with dendritic processes retain many or all of their innervating axons. Thus the presence or absence of ganglion cell dendrites alters competitive interactions among the axons that initially innervate a ganglion cell, probably by altering the availability of trophic support. Several axons can innervate a neuron when the neuron bears dendritic branches, whereas in the absence of dendrites there can be only a single input. This outcome cannot be simply a matter of the amount of available postsynaptic surface because even the cell body of a ganglion cell without dendrites is innervated by numerous synaptic contacts (all, however, from a single parent axon).

Additional evidence for the retrograde transsynaptic regulation of ganglion cell innervation comes from experiments where the

axons of ganglion cells are cut in adult animals^{23,24}. After axon section, most synapses made by preganglionic axons disappear from ganglion cell surfaces in a few days, but they are re-established when the injured axons regenerate. This reversible loss of connections implies that the synapses made by preganglionic axons are maintained by a signal that emanates from ganglion cells, a signal modulated in turn by the trophic relationship between ganglion cells and their peripheral targets.

Molecular explanation

The validity of the idea that the changing size and form of the mammalian body requires trophic feedback from target cells—and consequent adjustments of neuronal geometry and innervation—does not depend on the identification of a particular molecular signal that mediates these interactions. Nevertheless, one well-studied molecule, nerve growth factor (NGF), fits the criteria for such an intercellular signal in the sympathetic division of the autonomic neuron system.

NGF, discovered more than 30 years ago^{25,26}, determines the size of the neuronal populations that are sensitive to its action. A great deal of evidence (reviewed in ref. 27) now supports the view that NGF is synthesized by the peripheral targets of sympathetic ganglion cells and some classes of sensory neurons. These neurons have NGF receptors on their axon terminals, and retrogradely transport NGF to their cell bodies. In the presence of an adequate supply of target-derived NGF, the neurons sensitive to it survive; in the absence of NGF, they die.

The effects of NGF on the branching of sympathetic and sensory ganglion cells were apparent in the earliest experiments on NGF and provided the basis for the standard NGF bioassay^{25,26}. Further experiments showed that the administration of NGF to newborn rats leads to profuse axonal branching in the

Taken together, the effects of NGF and its anti-serum on the branching of sympathetic neurons suggest that NGF is at least a part of the trophic signal that links the form of neurons in sympathetic ganglia to the size of their peripheral targets.

The evidence we have discussed indicates that, in the peripheral autonomic nervous system, neuronal form and innervation are

The idea that the form and innervation of autonomic neurons are regulated by trophic interactions between these nerve cells and the target cells they innervate is an extension of the concept of trophic modulation of neuronal numbers. Indeed, we assume that the mechanisms that regulate the form and extent of neuronal processes are the same as, or variations of, the mechanisms that, at an earlier stage in development, help to define the size of nerve cell populations. At the molecular level, these retrograde trophic interactions seem to be based on the acquisition of specific molecules such as nerve growth factor. Because the trophic resources that autonomic neurons need are normally produced by cells in the targets they innervate, the axonal and dendritic morphology of ganglion cells and the inputs they receive can be continually shaped to meet the requirements of a changing body. This sculpting of neural connections by continuous trophic interactions may help to explain evolutionary as well as developmental changes in the organization of the nervous system.

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Quasiperiodicity in cataclysmic variable stars caused by solar-type magnetic cycles

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Cyclical variations of orbital periods, quiescent magnitudes and outburst intervals in the activity of cataclysmic variable binary stars are inter-related and are ascribed to variations in radii of the secondaries, caused by solar-type (sunspot) magnetic cycles. In the nova remnant DQ Herculis the observed variations in orbital period and quiescent magnitude are consistent with this mechanism. But accretion onto the white dwarf, from an accretion disk acquired from its companion, cannot explain the observed variation of the 71-second oscillations.

CATACLYSMIC variable stars comprise novae, dwarf novae and related stars that show eruptive behaviour. They are all binary stars, with one component a white dwarf and the other generally a more normal star, similar to the Sun, with a mass lying in the range $0.1\text{--}1.0 M_{\odot}$. Their orbital periods are among the shortest known for binary stars: most lie in the range 90 min to 0.5 day.

The normal star is strongly tidally distorted by its companion white dwarf, with the result that it loses gas from its atmosphere at the point nearest to its companion. The gas streams towards the white dwarf and goes into orbit around it, forming an accretion disk. The accretion disk is self-luminous as a result of viscous dissipation. A further source of luminosity is the shock region where the stream of gas impacts on the accretion disk.

Cyclical variations of orbital period have been securely established in several well observed eclipsing cataclysmic variable stars. The 29.0-yr apparent period¹ in the observed-calculated eclipse timings (O-C) of the nova-like system UX UMa was shown in later observations² to deviate significantly from strict repetition, suggesting that the cyclical behaviour is more correctly described as quasi-periodic. The nova-like RW Tri also shows^{1,3} cyclical variation of O-C, with a period of 8 or 14 yr. The dwarf nova U Gem possesses^{4,5} systematic variations in O-C, which we interpret here as an ~ 8 -yr quasiperiodicity. The nova remnant DQ Her has a 13.4-yr periodicity⁶. Another nova remnant, T Aur, has significant curvature in its O-C diagram, which, in the light of the quasi-sinusoidal variations observed in the above stars, is probably part of a ~ 23 -yr cycle⁵. EX Hya, a short-orbital-period dwarf nova, has a 19-yr orbital period cycle⁷ and a similar system, Z Cha, has O-C curvature^{8,9} that may also be part of a long-period cyclical variation.

The departure from strict periodicity in the orbital O-C diagrams rules out explanations such as apsidal motion in a slightly elliptical orbit¹⁰ or perturbations by a third body^{4,11}. The observed ranges of the O-C variations (all are ~ 0.001 d) are too large by at least an order of magnitude to be explained as the result of obvious orbital element variations which occur from mass exchange in these systems^{5,9}. Cyclical exchange of rotational and orbital angular momentum through interaction of the accretion disk¹² requires disk masses many orders of magnitude larger than those deduced from more direct observations of the disks. Spin-orbit coupling of a secondary star of variable radius¹³ requires a very short ($\ll 10$ -yr) timescale for tidal synchronization, which has been shown to be not available¹⁴. The timescales and amplitudes of the orbital period changes have therefore remained largely unexplained.

The recent discovery by Bianchini¹⁵ of periodic (or quasiperiodic) variations in mean brightness of some nova remnants and nova-like variables, and in the outburst intervals

of some dwarf novae, on timescales of 3–12 yr, suggests that the mechanism that produces the cyclical orbital period variations may have been detected in another guise. Bianchini suggests that solar-type magnetic cycles (similar to the 11- or 22-yr quasiperiodic sunspot cycle) occur in the secondary stars of the cataclysmic binary systems. Both the quiescent magnitudes and the outburst intervals are measures of the rate of mass loss from the secondaries¹⁶. That mass transfer could be significantly affected by solar-type cycles can be seen in the following way. Thomas¹⁷ has argued that, because of the varying number of magnetic flux tubes¹⁸ (and hence, magnetic pressure support) over a solar cycle, the radius of the Sun, R , should vary over the cycle by $\Delta R/R \sim 5 \times 10^{-4}$ or more. Gilliland¹⁹ computed solar models in which, with appropriate variations of magnetic pressure, these radius variations were confirmed. On the other hand, observations appear to show radius variations $\Delta R/R \sim 1 \times 10^{-4}$, anticorrelated with field strength in the solar cycle²⁰, which may result from the reduction in radius that occurs when convection is more inhibited by higher magnetic fields. Given that in the tidally synchronized, and thus rapidly rotating, secondary stars of cataclysmic variables, dynamo generation of magnetic fields should be as successful as in the Sun—at least for stars with some radiative core in which to anchor the field²¹—similar radius variations and timescales would not be surprising.

The scale height of the atmosphere of a lower-main-sequence (LMS) star is²²

$$H \approx 2.22 \times 10^7 \left(\frac{M}{M_{\odot}} \right)^{0.854} \text{ cm} \quad (1)$$

where M is the mass of the star. Patterson²³ finds that the Roche-lobe-filling LMS stars in cataclysmic variables possess a mass-radius relationship of the form

$$R/R_{\odot} \approx \left(\frac{M}{M_{\odot}} \right)^{0.88} \quad (2)$$

Equations (1) and (2) give

$$H/R \approx 3.2 \times 10^{-4} \quad (3)$$

for all LMS secondaries.

Following Osaki²⁴ the rate of mass transfer $\dot{M}$ (here taken to be positive), corresponding to a radius $R + \Delta R$ of the secondary star, is

$$\dot{M} = \dot{M}_0 e^{\Delta R/H} \quad (4)$$

where $\dot{M}_0$ is the rate for the undisturbed star ($\Delta R = 0$). (Use of an alternative formulation, involving physical conditions near

the inner lagrangian point, where H is redefined, leads to exponents within a factor of two of the above²².)

Equations (3) and (4) give

$$\dot{M} = \dot{M}_0 e^{3.1 \times 10^3 \Delta R / R} \quad (5)$$

Orbital period variations

The tidally distorted secondaries in cataclysmic variables cause an asymmetrical gravitational potential which, if their orbits were elliptical, would lead to detectable apsidal motion^{10,25}. Even if the orbits are circular, the observed orbital period P_{obs} is not the same as the period, P_{orb} , that would obtain for spherically symmetrical stars.

For a synchronously rotating secondary, the period U of apsidal motion is given by²⁶

$$\frac{P_{\text{orb}}}{U} = k_2 \left(1 + \frac{16}{q} \right) \left(\frac{R}{a} \right)^5 \quad (6)$$

where k_2 is the apsidal coefficient, $q = M_2/M_1$ is the mass ratio of the secondary to the primary and a is the distance between the two components. The observed orbital period is given by $P_{\text{obs}}^{-1} = P_{\text{orb}}^{-1} + U^{-1}$ or, as $U \gg P_{\text{orb}}$,

$$P_{\text{obs}} = P_{\text{orb}} \left[1 - k_2 \left(1 + \frac{16}{q} \right) \left(\frac{R}{a} \right)^5 \right] \quad (7)$$

The outer convection zones of stars with $M \leq M_{\odot}$ are sufficiently deep that they encompass that part of the tidally distorted envelope that is predominantly responsible for the gravitational quadrupole moment. An expansion of the convection zone by increase of magnetic pressure therefore produces an increase of quadrupole moment, the effect of which is governed by equation (6). But as the tidal interaction timescale is considerably larger^{14,27} than the periods of the changes of secondary radii, spin-orbit interaction is negligible and the orbit must maintain constant angular momentum. If the orbital period is written as $P_{\text{obs}} \propto a^{3/2}(1-\delta)$, then the orbital angular momentum $L \propto a^{1/2}(1+\delta)$.

The apsidal coefficient k_2 is approximately proportional to the seventh moment of inertia of the star²⁶:

$$k_2 \propto \int_0^1 \rho(b) b^7 db \quad (8)$$

where $b = r/R$ is the fractional distance from the centre of the star and ρ is the density. Expansion of the convection zone moves material outwards and increases the moments of inertia of the star. From approximations for $\rho(b)$, or on dimensional arguments, we find $dk_2/k_2 = 5 dR/R$.

With this relationship and the condition $dL/dR = 0$, differentiation of equation (7) gives

$$\frac{\Delta P_{\text{obs}}}{P_{\text{obs}}} = -40k_2 \left(1 + \frac{16}{q} \right) \left(\frac{R}{a} \right)^5 \frac{\Delta R}{R}$$

or, because $q \leq 1$,

$$\approx -640 \frac{k_2}{q} \left(\frac{R}{a} \right)^5 \frac{\Delta R}{R} \quad (9)$$

Using the relationship²⁸

$$\frac{R}{a} = 0.459 \left(\frac{q}{1+q} \right)^{1/3} \quad (10)$$

we have

$$\frac{\Delta P_{\text{obs}}}{P_{\text{obs}}} = -13.0k_2 q^{2/3} (1+q)^{-5/3} \frac{\Delta R}{R} \quad (11)$$

If R undergoes a cyclical variation of the form

$$\frac{\Delta R}{R} = A \sin \left(\frac{2\pi t}{W} \right) \quad (12)$$

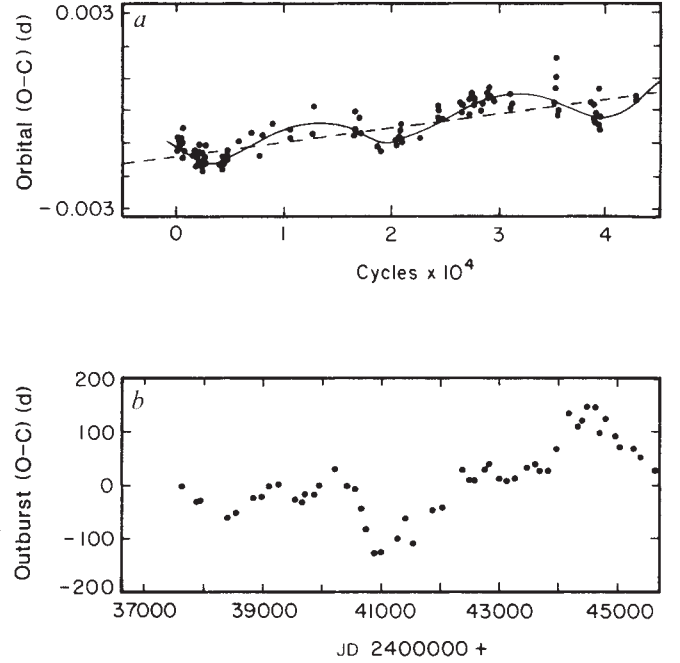


Fig. 1 O-C diagrams of *a*, the times of eclipse of U Geminorum as a function of the number of orbital cycles; and *b*, the outburst times as a function of Julian date. Both diagrams have the same time axis and both have been adjusted for an abrupt change of mean interval between outbursts (see text). The dashed line in *a* indicates an overall slowly increasing O-C, and the solid line is the superimposed cyclical variation. Dots are the observational values.

then the O-C diagram of, for example, the times of eclipse minima will vary as

$$(O-C)_{\text{orbital}} = 13.0k_2 q^{2/3} (1+q)^{-5/3} \frac{AW}{2\pi} \cos \left(\frac{2\pi t}{W} \right) \quad (13)$$

This will be superimposed on the secular change of O-C caused by the mass transfer process. As the variations (12) of radius cause modulation of $\dot{M}$ through equation (5) (from equations (7) and (10), and the condition $L = \text{constant}$, it can be shown that the change in radius of the Roche lobe, consequent on the change in a required to conserve angular momentum is small compared with the change ΔR in radius), the secular change will itself be modulated with period W . For small changes $\Delta R/R \approx 10^{-4}$, the amplitude of this variation is

$$\sim 1.9 \times 10^{-5} (1-q) \frac{AW}{2\pi} \left(\frac{M_{\odot}}{M} \right) \left(\frac{\dot{M}}{10^{-9} M_{\odot} \text{ yr}^{-1}} \right) T(\text{yr}) \quad (14)$$

for the conservative case²⁹. For $\dot{M} \leq 3 \times 10^{-8} M_{\odot} \text{ yr}^{-1}$ and $T \leq 50 \text{ yr}$, which covers all observed cases, the amplitude (14) is small compared with that in equation (13). Therefore, the changes in R affect P_{obs} more through variations of the quadrupole moment than through the dynamical effects of variations of $\dot{M}$.

The apsidal coefficient k_2 for main sequence stars is typically ~ 0.01 at $M = 1 M_{\odot}$, rising rapidly to 0.143 at $0.4 M_{\odot}$, at which value it remains for lower masses because the stars are fully convective and resemble $n = 1.5$ polytropes^{25,30}. At $M = 0.60 M_{\odot}$, $k_2 = 0.0565$ (ref. 31). In the secondaries of cataclysmic variables, rapid mass loss can drive their structure out of thermal equilibrium (see discussion in ref. 32), increasing the depth of the outer convection zone and increasing the radius of the star. To evaluate the effect of this on k_2 requires detailed models.

Taking $k_2 = 0.05$, $q = 0.5$ and $W = 7 \text{ yr}$, equation (13) predicts that the orbital O-C diagram will show a modulation with

peak-to-peak range of $\sim 170 A$ days. A radius variation with amplitude $A \sim 5 \times 10^{-6}$ is therefore sufficient to account for the observed modulations in the O-C diagrams.

The theory of orbital periods given here resembles that proposed by Applegate and Patterson¹⁴, but does not require a variable global magnetic field topology—variations of mean radius alone are able to produce period changes. But if magnetic flux tubes concentrate towards the equatorial plane, variations in their number may increase the equatorial radius more than the polar radius, which would enhance the quadrupole moment.

Mass transfer variations

Variations in ΔR modulate $\dot{M}$ through the effect of equation (5). Variations in $\dot{M}$ can be detected through changes in the bright spot and accretion disk luminosities and through variations in the intervals between outbursts in dwarf novae¹⁶.

In the disk instability model of dwarf nova outbursts³³, mass is stored in the disk until a critical point is reached at which viscosity increases greatly and the accumulated mass falls onto the white dwarf primary component. If the amount of mass that must be stored before it triggers the instability is roughly constant, then

$$(\dot{M} - \dot{M}_d)\tau \approx \text{constant} \quad (15)$$

where $\dot{M}_d$ is the rate of mass transfer through the quiescent accretion disk and τ is the interval between outbursts. In general $\dot{M}_d \ll \dot{M}$ in dwarf novae¹⁶ (but in any case, it is possible that the viscosity in the disk is generated by internal waves excited by noise from the impact between stream and disk (E. T. Vishniac and P. Diamond, preprint), in which case $\dot{M}_d \propto \dot{M}$) and so (15) implies

$$\dot{M} \propto \tau^{-1} \quad (16)$$

or

$$\frac{\tau_1}{\tau_2} \propto \exp(3.1 \times 10^3 (\Delta R_2 - \Delta R_1)/R) = \exp(-3.1 \times 10^3 \Delta R/R) \quad (17)$$

from equation (5).

The range of visual luminosity resulting from variations in $\dot{M}$ is not accurately inferrable. The disk has an approximate absolute magnitude¹⁶ $M_v \approx -1.85 \log \dot{M}_d + \text{constant}$. The bright-spot component, being of lower effective temperature, may vary nearer to $M_v \propto -2.5 \log \dot{M}$. We adopt, as a compromise, an apparent magnitude variation

$$\Delta m_v \approx -2.0 \log \frac{\dot{M}_1}{\dot{M}_2} \quad (18)$$

$$\approx 2.7 \times 10^3 \frac{\Delta R_2 - \Delta R_1}{R} = -2.7 \times 10^3 \frac{\Delta R}{R} \quad (19)$$

from equation (5), where $\dot{M}_1$ and $\dot{M}_2$ are the mass transfer rates corresponding to ΔR_1 and ΔR_2 .

Equations (13), (17) and (19) provide independent estimates of $\Delta R/R$. However, whereas (17) and (19) are based on observational properties that measure directly the energetics controlled by the rate at which mass is overflowing the Roche lobe, equation (13) gives $\Delta R/R$ by a dynamical method which is sensitive only to changes of structure in the region where the star's quadrupole moment is determined. For example, if variations of magnetic flux during the magnetic cycle occurred only near the photosphere, this would affect $\dot{M}$ but would leave the quadrupole moment, and hence P_{obs} , almost unchanged. Clearly we can relate the two methods of estimating $\Delta R/R$ only if we have a model for the distribution of flux tubes in the star and for the way that this distribution changes during a magnetic cycle.

We shall see below that the observations require $(\Delta R/R)_{\text{energetics}} = \alpha (\Delta R/R)_{\text{dynamical}}$ with $\alpha \approx 10$.

SS Cygni. Bianchini¹⁵ finds that the outburst intervals in the dwarf nova SS Cyg have a cyclical variation with a period of

7.3 yr and range from 42 to 58 d. From equation (17) the total radius change implied by this variation in τ is $\Delta R/R \approx 1.0 \times 10^{-4}$. Substituted into equation (19), this gives an expected range $\Delta m_v = 0.27$ in the quiescent disk and/or bright spot. A. Kiplinger (private communication) has Fourier analysed the quiescent magnitudes of SS Cyg and independently finds a 7.3-yr period. The times when the outbursts occur more frequently correspond to the times when the quiescent magnitudes are brightest, in agreement with equations (17) and (19). Kiplinger finds a peak-to-peak range of 0.15 mag for the 7.3-yr quiescent magnitude cycle. As 60% of the quiescent brightness of SS Cyg is contributed by the secondary³⁴, the estimate $\Delta m_v = 0.27$ given above must be reduced to $\Delta m_v = 0.17$ for the expected range of apparent magnitude. There is therefore excellent agreement between the two independent measures of $\Delta R/R$.

U Geminorum. Bianchini¹⁵ finds that the outburst intervals of the dwarf nova U Gem vary with a period of 6.9 yr and range from 92 to 132 d. This ratio is similar to that found in SS Cyg and implies a comparable range of $\dot{M}$ and ΔR . The cyclical variation of τ reported by Bianchini shows as an equally strong periodicity in the O-C diagram of its outbursts³⁵ during the years 1890 to ~ 1926 ; after 1922 there was an abrupt increase of ~ 26 days in average τ and the cycles are less easy to identify. The mean period of the prominent cycles during 1890–1921 is 7.3 yr; a similar period is detectable in the diagram for 1938–61, but of lower amplitude and shifted in phase relative to the 1890–1921 cycles. This probably accounts for the slightly different mean period measured by Bianchini.

The orbital O-C diagram, found from eclipse timings of U Gem, shows systematic variations which have been interpreted as an ~ 19 -yr cycle^{4,5}. In the light of the foregoing discussion, however, the diagram requires a new interpretation.

Equations (5), (11) and (16), and the comparative factor α , combine to give

$$\frac{d\tau}{\tau} = 2.4 \times 10^2 \frac{\alpha}{k_2} \frac{(1+q)^{5/3}}{q^{2/3}} \frac{\Delta P_{\text{obs}}}{P_{\text{obs}}} \quad (20)$$

which shows that P_{obs} should appear to vary as a function of τ . The orbital O-C diagram given by Beuermann and Pakull⁵ covers the period 1961–82. From 1960 until 1969 the outburst O-C diagram³⁵ shows that U Gem had outbursts with a mean interval of ~ 98 d, after which there was an abrupt increase to 121 d. Taking $k_2 = 0.056$ and $q = 0.475$ for U Gem³⁶, and anticipating $\alpha = 10$, this 23% decrease in $\dot{M}$ implies (equations (10) and (17)) an increase $\Delta P_{\text{obs}}/P_{\text{obs}} = 1.5 \times 10^{-6}$ at the end of 1979, which transforms into a negative slope of -2.6×10^{-7} d per cycle in the orbital O-C diagram during 1961–69.

Removal of the pre-1969 trend produces the modified O-C diagram shown in Fig. 1a. The previously discerned^{4,5} 19-yr period was caused by large O-C values near the beginning of the observational record. With these removed we can now see an ~ 8 -yr cyclical variation (solid line) superimposed on a slowly increasing O-C (dashed line). The latter merely means that the P_{obs} deduced by Beuermann and Pakull must be increased now that the effect of the change in τ in 1969 has been removed. The revised period, $P_{\text{obs}} = 0.176906226$ d, is that corresponding to $\tau = 121$ d.

As an eclipsing system in which variations of both P_{obs} and τ have been observed, U Gem provides a valuable source with which to compare the theory developed above. Through equation (20) we would expect that the orbital and outburst O-C diagrams should vary in phase with each other. To compare the outburst O-C diagram with Fig. 1a, the times of outburst³⁵ in U Gem were analysed for the period 1961–82. To produce compatibility with Fig. 1a, the O-Cs were calculated relative to $\tau = 98$ d for 1961–69 and relative to 121 d thereafter. Unfortunately, U Gem lies near the ecliptic, so there are gaps in the observations of outbursts. I agree with Cook³⁵ that the assumed outburst 406 probably never happened, and I suspect also that

assumed outburst 443 did not exist. With these two eliminated, the O-C diagram is as shown in Fig. 1b. With the exception of the final one-fifth of the time covered, Fig. 1a and b are reasonable replicas of each other. Better agreement in the final sections of the diagrams can be produced if there was an outburst missed between listed outbursts 436 and 437—at a time when U Gem was covered by the Sun. This would have the effect of displacing the final twelve points of the outburst O-C diagram downwards by 121 d.

Despite some imperfections, the morphology of the variations in Fig. 1a and b are sufficiently similar to furnish an estimate of the scale factor relating them: Fig. 1b transforms to Fig. 1a after multiplication by 8×10^{-6} . From equation (20), and adopting the parameters for U Gem listed above, we find $\alpha \approx 9.5$.

DQ Herculis. Patterson *et al.*⁶ found a 13.4-yr cycle in the orbital period of the nova remnant DQ Her. Their ephemeris shows that maximum ΔP_{obs} (corresponding to maximum positive slope in the orbital O-C diagram) occurred on 1976.4.

Variations in the brightness of DQ Her have been noted³⁷ and were thought to be associated with a decline of the system since its eruption in 1934. But Dmitrienko³⁸ has noted recently that in 1982, DQ Her had recovered the brightness it had when first observed photoelectrically by Walker³⁹ in 1954. It was also bright around 1970, and faded over the following seven years⁶, as it did also after its 1982 maximum³⁸. There is, therefore, clear evidence for a ~14-yr cycle in the brightness of DQ Her, with a minimum occurring in about 1976–77, in agreement with the time of maximum $\Delta P_{\text{obs}}/P_{\text{obs}}$ (and hence, most negative ΔR (from equation (11) and smallest $\dot{M}$). The total range of the apparent magnitude variations is $\Delta m_v \approx 0.6$, which should be readily detectable in archival photographic records. This is the same range as already detected in nova remnants GK Per and Q Cyg¹⁵.

From equation (18), $\Delta m_v = 0.6$ gives an amplitude $A = 1.1 \times 10^{-4}$. The orbital O-C amplitude⁶ is 0.0008 d, which gives $A/\alpha = 4.4 \times 10^{-6}$, and hence $\alpha = 25$, in DQ Her. This is considerably larger than what was found in U Gem. The physical conditions are, however, very different in the two stars: U Gem is an unusually faint dwarf nova¹⁶ with $\dot{M} \approx 10^{11} M_{\odot} \text{ yr}^{-1}$, whereas DQ Her has $\dot{M} \approx 10^{-8} M_{\odot} \text{ yr}^{-1}$, probably because the atmosphere of the secondary is heated by the hot white dwarf remnant of the 1934 eruption²², which may alter the relationship between $\dot{M}$ and ΔR .

The mass transferred through the disk is accreted by the white dwarf primary. The angular momentum brought by this accreted material spins up the white dwarf, an effect which has observable consequences in the case of DQ Her, for which its 71.07-s rotation period P_{rot} is measured⁴⁰ to be decreasing at a rate $\dot{P}_{\text{rot}} = -8.1 \times 10^{-13} \text{ s s}^{-1}$. Variations of $\dot{M}$ caused by magnetic cycle effects in the secondary should therefore modulate P (the accretion disk in DQ Her is in the viscous high state, so there is rapid transference of material from the outer to the inner regions of the disk). Systematic departures from a smooth ephemeris in the 71-s rotational O-C diagram have been observed by Balachandran *et al.*⁴⁰.

The theory of accretion torques⁴¹ indicates that $-\dot{P}_{\text{rot}} \propto \dot{M}^{6/7}$. Equations (12) and (19) then lead to

$$(O-C)_{\text{rotational}} = \frac{6}{7} \frac{\delta m_v}{2.0 \log_{10} e} \left(\frac{W}{2\pi} \right)^2 \frac{\dot{P}_{\text{rot}}}{P_{\text{rot}}} \bigg|_{t=0} \sin \left(\frac{2\pi t}{W} \right) \quad (21)$$

$$= -51.6 \delta m_v \sin \left(\frac{2\pi t}{W} \right) \text{ s for DQ Her} \quad (22)$$

where δm_v is the amplitude (assumed small) of the variations of apparent magnitude and $(O-C)_{\text{rotational}}$ is relative to a quadratic ephemeris (that is, after removal of the secular change in P_{rot}). The negative sign in equation (22) indicates that $(O-C)_{\text{rotational}}$ and m_v are expected to vary in antiphase. For DQ Her, $\delta m_v \approx 0.3$ mag, and we expect the values of $(O-C)_{\text{rotational}}$ to vary over the range ± 15 s with period $W = 13.4$ yr.

Turning to the observations^{4,40}, the spread of $(O-C)_{\text{rotational}}$ does indeed cover the range $\sim \pm 15$ s, but there is a contradiction in the phasing. If Fig. 1 of ref. 40 is redrawn modulo the period $W = 13.4$ yr, a plausible but very noisy cyclic variation is found, with minimum $(O-C)_{\text{rotational}}$ near 1977. This is the time of maximum disk brightness (see above), whereas according to equation (22), minimum $(O-C)_{\text{rotational}}$ should occur at maximum m_v , that is, at minimum disk brightness. The correlation between bright m_v and minimum $(O-C)_{\text{rotational}}$ is, of course, inherent in the observations and not dependent on any theory. In physical terms, because $-\dot{P}_{\text{rot}} \propto \dot{M}^{6/7}$ we would expect maximum $\dot{M}$ (and therefore brightest disk) to correspond to times of most rapid decrease of the 71-s periodicity, but the observations show that at this time $(O-C)_{\text{rotational}}$ is already at its most negative value and is about to start increasing.

The same phase discordance is seen between $(O-C)_{\text{orbital}}$ and $(O-C)_{\text{rotational}}$: equations (13) and (21) require the phases to differ by $\pi/2$, whereas observationally they differ by $3\pi/2$.

It is perplexing that whereas the theory of accretion torques gives a correct estimate of $\dot{P}$ for DQ Her⁴¹, the response of $\dot{P}$ to changes in $\dot{M}$ is the reverse of prediction. This, however, is a problem in accretion theory and is not directly relevant to the present topic of magnetic cycles in the secondary stars.

A ~14-yr cyclic variation with a range $\Delta m_v = 0.2$ is seen⁴² in the decline portion of the outburst light curve of Nova Pictoris 1942 and probably represents a brightness modulation of similar amplitude to that in DQ Her, reduced by some residual (unmodulated) luminosity from the hot nova remnant.

Orbital periods

Short-orbital-period systems. Fully convective stars have $k_2 = 0.143$, and the sensitivity of the orbital O-C diagram (equation (13)) to variations of R is increased threefold over what we have seen for U Gem, SS Cyg, and DQ Her. Fully convective secondaries are thought to occur in cataclysmic variables with $P_{\text{orb}} \leq 2$ h (see, for example, the discussion by Verbunt and Zwaan³²), but explanations of the 'orbital period gap' ($2 \leq P_{\text{orb}} \leq 3$ h) require that the magnetic braking mechanism driving the mass transfer process either cease or be very much reduced in fully convective stars. If there were no magnetic braking at all below the period gap, only gravitational radiation would be available to drive mass transfer. But many cataclysmic variables below the period gap have $\dot{M}$ considerably larger than that predicted for gravitational radiation loss¹⁶, suggesting that their secondaries do in fact possess magnetic fields. (Radio emission from the short period system V384 Cen has been interpreted as possibly arising in the secondary star⁴³.) This conclusion is strengthened by the presence of an ~19-yr cyclical variation of period in EX Hya⁷: if the ~10-yr period variations found above the period gap are caused by magnetic cycles then so presumably are those below the gap. In EX Hya ($P_{\text{orb}} = 1.64$ h) the 0.00025-d amplitude of the orbital O-C variations, through equation (13) and with $q = 0.15$ (this is not well determined, but equation (13) is insensitive to the choice of q) and $k_2 = 0.14$, implies $A = 5.4 \times 10^{-7}$. This is an order of magnitude smaller than the values of A for stars above the period gap and is compatible with the expected lower magnetic field strengths.

Long-orbital-period systems. For $M \geq 0.75 M_{\odot}$, k_2 diminishes rapidly, and for Roche-lobe-filling LMS stars²³ $P_{\text{orb}} \propto M^{0.82}$, so the effects of variable R will become difficult to detect for $P \geq 7$ h. There are few eclipsing cataclysmic variables at these longer periods, so it is not possible to check this prediction. Note, however, that the early suspicion²⁹ of orbital period variations in EM Cyg ($P_{\text{orb}} = 6.96$ h) has disappeared now that a longer baseline of observations is available⁵.

Discussion

Table 1 summarizes the known properties of cataclysmic variables possessing cyclical variations in their orbital periods, outburst intervals or quiescent magnitudes. Many single stars

Table 1 Long-term periodicities in cataclysmic variables

Star	Type*	P_{orb} (h)	W (yr)	A (energetics)	A (dynamical)	Refs
EX Hya	DN	1.64	19		5.4×10^{-7}	7
TT Ari	NL	3.30	12.6			15
RR Pic	N	3.48	14	$> 6 \times 10^{-5}$		42
U Gem	DN	4.17	6.9	5.1×10^{-5}	5.4×10^{-6}	4, 5, 15
DQ Her	N	4.65	13.4	1.1×10^{-4}	4.4×10^{-6}	6
UX UMa	NL	4.72	29.0		4.8×10^{-6}	1, 2
T Aur	N	4.91	23			5
RW Tri	NL	5.57	8 or 14		$8.0 \text{ or } 4.6 \times 10^{-6}$	3
SS Cyg	DN	6.63	7.3	5.0×10^{-5}		15
GK Per	N	48	6.9	1.3×10^{-4}		15
Q Cyg	N	—	6.6	1.1×10^{-4}		15
V841 Oph	N	—	3.3			15

* DN = dwarf nova, N = nova, NL = nova-like.

show cyclic behaviour of magnetic field strength indicators with periods W in the range 3–16 yr (the upper limit of which is strongly influenced by the lengths of available data), which show no correlation with rotation period or mass of star⁴⁴. The cycles discerned in the cataclysmic variables certainly cover the same range of W and are able to extend to longer periods because of the availability of longer term observations. They also show no clear correlation of W with P_{orb} (that is, with rotation period or mass, both of which are essentially determined by P_{orb}). However, the cataclysmic variables extend knowledge of values of W down to $P_{\text{orb}} \sim 1$ –0.5 h and of M to $\sim 0.1 M_{\odot}$, and to much greater values of W/P_{orb} (up to 10^5 , as opposed to ~ 200 seen in single stars).

The lengths of the cycles, their quasiperiodic nature and the values of $\Delta R/R \approx 1 \times 10^{-4}$ derived from the mass overflow-sensitive technique, are all in accord with the hypothesis that they are solar-type magnetic cycles. Indeed, the variable amplitude of modulation, visible in the U Gem outburst O–C diagram³⁵ and present in the SS Cyg quiescent magnitudes (A. Kiplinger, private communication), resembles the variation of the sunspot cycle and the occurrence of periods of inactivity (such as the Maunder minimum)⁴⁵. The theoretical field strengths required for the magnetic braking mechanism are much larger than the average photospheric field in the Sun and imply greater chromospheric and coronal activity in the secondaries of cataclysmic variables. Direct evidence of the presence of magnetic fields is, however, sparse. X-ray emission and optical and ultraviolet emission lines in a cataclysmic variable are generated principally in the accretion disk and its boundary with the white dwarf, and these in general overpower any emissions from the secondary. But the correlation between surface flux of X-ray emission and rotation period for LMS stars⁴⁶, when extrapolated to the shorter rotation periods appropriate to cataclysmic variables, is able to account for the 0.6-keV invariant (during outbursts) component of X-rays in SS Cyg and all of the X-rays from AE Aqr⁴⁷. The latter is particularly significant because, at $P_{\text{orb}} = 9.9$ h, the secondary component dominates the spectrum in the visible region, and, unlike in other systems, strong emission lines from the secondary are readily detectable⁴⁸. Therefore, at least in this cataclysmic binary for which the secondary is conspicuous, it appears to be magnetically active at a level compatible with expectation. AE Aqr is a fruitful system for long-term monitoring: its X-ray flux, radio emission⁴⁹ and emission line strengths are indicators of magnetic field strength, and its quiescent magnitude will monitor changes in mass transfer. Unfortunately, it is not an eclipsing system; in any case, the mass of its secondary is high enough to give $k_2 \approx 10^{-2}$ and therefore expectedly small values of $\Delta P_{\text{obs}}/P_{\text{obs}}$.

Visual observations of cataclysmic variable stars often extend back 50 or even 100 years. Archival plate material is available that gives coverage of the brighter of the more recently discovered systems back to the early years of this century. All of

these data can be analysed for cyclical behaviour, which may add substantially (and without waiting for accumulation of further observations of single stars) to knowledge of magnetic cycles in LMS stars. The possibility of finding longer periods, similar to the apparent 76-yr period in solar radius measurements²⁰, is also opened up. For example, the outburst O–C diagram³⁵ of U Gem possesses 2.5 cycles with a possible ~ 47 -yr period, as well as the 7-yr period. The ratio of these is similar to the ratio of the 76- and 11-yr cycles of the Sun.

The abrupt change in U Gem from $\tau \approx 98$ d during 1855–1922 to $\tau \approx 124$ d in 1922–1985 occurred at the end of one of the ~ 47 -yr cycles. Such a change implies a substantial adjustment to different mean rate of mass transfer. This could be the result of an even longer cycle of magnetic activity, but on timescales of ~ 100 yr changes in $\dot{M}$ are also expected in the cyclical evolution theory of cataclysmic variables^{16,22}.

If our interpretation given to orbital period variations is correct, the availability of two estimates of $\Delta R/R$, one dependent on what is happening near the photosphere and the other on conditions deep in the convection zone, provides a quantitative means of comparing magnetic cycle theory with observation.

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Restoration of excitation-contraction coupling and slow calcium current in dysgenic muscle by dihydropyridine receptor complementary DNA

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Microinjection of an expression plasmid that carries complementary DNA encoding the receptor for dihydropyridine calcium channel blockers of skeletal muscle restores both excitation-contraction coupling and slow calcium current in cultured skeletal muscle cells from mice with muscular dysgenesis. This suggests that the dihydropyridine receptor in the transverse tubule membrane of skeletal muscle functions both as the voltage sensor for excitation-contraction coupling and as the slow calcium channel.

THROUGH a series of events, collectively termed excitation-contraction (E-C) coupling, electrical stimulation causes a muscle cell to contract. In vertebrate skeletal muscle, it is known that the contraction is caused by a transient rise in the intracellular Ca^{2+} concentration, a result of the release of Ca^{2+} from a wholly intracellular store in the sarcoplasmic reticulum (SR), and that the release of Ca^{2+} from the SR is caused by depolarization of transverse tubules (T tubules)^{1,2}. These are invaginations of the surface membrane that penetrate into the muscle interior. Although it has been suggested that the communication between T tubules and SR occurs at anatomically recognizable structures called triads, the molecular basis for the transduction of the electrical signal into the release of Ca^{2+} is not yet understood. One hypothesis is that the release of Ca^{2+} from the SR is controlled by a voltage sensor in the T-tubule membrane, that depolarization causes a molecular rearrangement of the voltage sensor and that this molecular rearrangement produces a characteristic intramembrane charge movement that has been measured in skeletal muscle³. Identification of the molecule that serves as the postulated voltage sensor would be essential for understanding the mechanism of E-C coupling.

The T-tubule system of skeletal muscle is known to contain a receptor for 1,4-dihydropyridine derivatives, a class of organic compounds that specifically modulate slow L-type calcium channels in skeletal muscle^{4,5} as well as in neurons⁶ and cardiac cells⁷. A number of recent observations provide evidence in support of the hypothesis that the dihydropyridine (DHP) receptor in skeletal muscle is the T-tubular voltage sensor for E-C coupling. Most significantly, DHP simultaneously alters intramembrane charge movement and the release of Ca^{2+} from the SR (ref. 8). Moreover, the primary structure of the skeletal muscle DHP receptor⁹, deduced from cloning and sequence analysis of the cDNA, reveals that this receptor is homologous with the voltage-gated sodium channel¹⁰⁻¹² and contains a putative voltage sensor segment (S4) in each of four internal repeats. This structural similarity indicates that the T-tubular DHP receptor may have a dual function, acting both as the voltage sensor for E-C coupling and as a calcium channel⁹. However, there

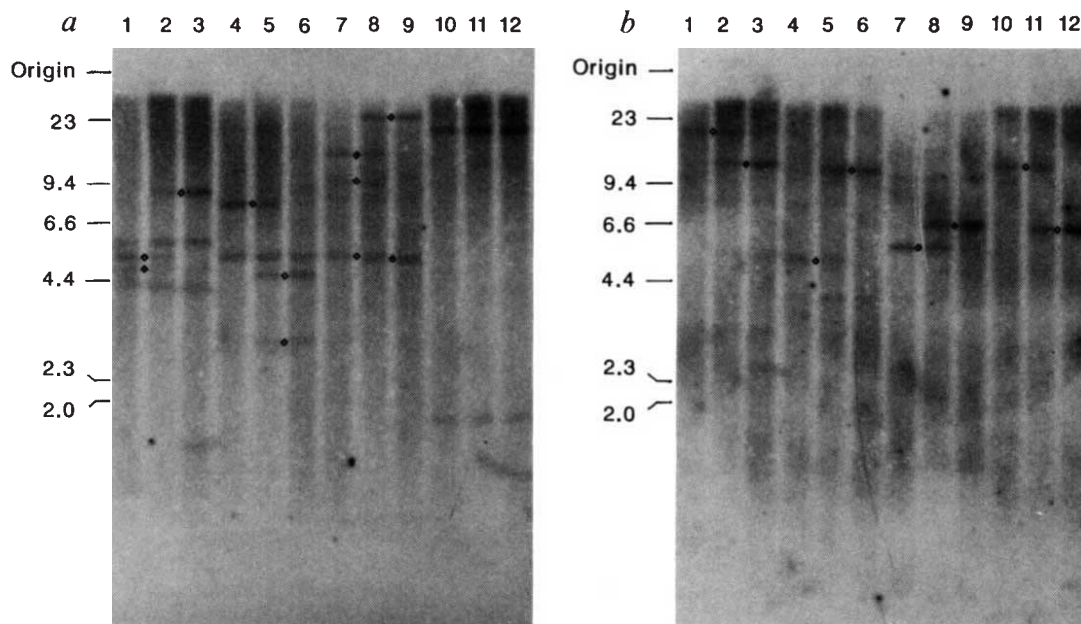
has not yet been a direct demonstration that the protein encoded by the DHP receptor cDNA functions as an essential component of E-C coupling and/or as a calcium channel. To address this problem, we have now conducted studies on mice with muscular dysgenesis, a fatal autosomal recessive mutation¹³ that is expressed in skeletal muscle as a failure of E-C coupling^{14,15} and absence of the DHP-sensitive calcium current I_{slow} (refs 16, 17). The results obtained show that both these functional defects in dysgenic mouse muscle can be restored by microinjection of an expression plasmid carrying the rabbit skeletal muscle DHP receptor cDNA. This result, together with blot hybridization analysis of genomic DNA and skeletal muscle RNA, suggests that the muscular dysgenesis mutation alters the structural gene for the skeletal muscle DHP receptor.

Analysis of genomic DNA

To investigate whether the muscular dysgenesis mutation represents an alteration in the DHP receptor gene, we performed restriction endonuclease analysis of genomic DNA. DNA preparations, obtained from livers of newborn mice that were homozygous (*mdg/mdg*) for the muscular dysgenesis mutation and of adult mice that were normal (+/+) or heterozygous (+/*mdg*), were subjected to blot-hybridization analysis. Four different restriction endonucleases (*Apa*I, *Bam*HI, *Eco*RI and *Kpn*I) and nine probes (probes 1-9; see Fig. 1 legend and Fig. 3a) derived from different regions of the rabbit skeletal muscle DHP receptor cDNA were used. Figure 1 shows the results obtained with probe 2(a) and probe 6(b). The DNA of an *mdg/mdg* mouse has hybridizable restriction fragments that are different in size from those derived from the DNA of a +/+ mouse (indicated by diamonds). The DNA of a +/*mdg* mouse yields both the normal and altered restriction fragments, as expected if the heterozygous mouse possesses one normal and one mutant copy of the DHP receptor gene. The disparity in restriction fragments between the +/+ and the *mdg/mdg* mouse revealed by probe 2 is different from that revealed by probe 6.

Probe 1, which included a region 5' to that targeted by probe 2, detected some of the same *Eco*RI fragments that were shown

Fig. 1 Autoradiograms of blot hybridization analysis of genomic DNA from normal and muscular dysgenic mice. Samples (2 μ g each) of DNA extracted from the livers of a normal (+/+) adult mouse (lanes 1, 4, 7 and 10), a heterozygous (+/mdg) adult mouse (lanes 2, 5, 8 and 11) and a dysgenic (mdg/mdg) newborn mouse (lanes 3, 6, 9 and 12) were analysed by the procedure of Southern⁴¹. The DNA samples were digested with the restriction endonuclease *Apa*I (lanes 1–3), *Bam*HI (lanes 4–6), *Eco*RI (lanes 7–9), or *Kpn*I (lanes 10–12). Nine different probes (probes 1–9 given below) were used, but data are shown only for probe 2 (a) and probe 6 (b). Restriction fragments that differ in size between the DNAs from the +/+ and the mdg/mdg mouse are indicated by diamonds; the left and the right vertex of each diamond point to a common fragment between +/+ and +/mdg or between +/mdg and mdg/mdg. The nine probes (labelled⁴² with [α -³²P]dCTP; specific activity, $\sim 1 \times 10^9$ c.p.m. μ g⁻¹), derived from the rabbit skeletal muscle DHP receptor cDNA⁹, were as follows: probe 1, *Nhe*I(-159)/*Pst*I(111) fragment (from the plasmid pCAC8 given below); probe 2, *Hind*III(5'-terminal linker)/*Eco*RI(1,007) fragment; probe 3, *Apa*I(804)/*Apa*I(3,098) fragment; probe 4, *Eco*RI(1,007)/*Sac*I(5,315) fragment; probe 5, *Pvu*II(1,075)/*Pvu*II(2,216) fragment; probe 6, *Pvu*II(2,216)/*Pvu*II(2,868) fragment; probe 7, *Apa*I(3,098)/*Apa*I(4,187) fragment; probe 8, *Apa*I(4,187)/*Apa*I(5,131) fragment; and probe 9, *Bgl*II(4,488)/*Hind*III(3'-terminal linker) fragment (see Fig. 3a). pCAC8 was constructed by ligation of the 0.21-kilobase pair (kb) *Pst*I(vector)/*Sac*II(-41) fragment from clone pCCH704 (ref. 9), the *Sac*II(-41)/*Nco*I(-1) and *Nco*I(-1)/*Aat*II(483) fragments from clone pCCH303 (ref. 9) and the 3.7-kb *Pst*I/*Aat*II fragment from the plasmid pBR322 (see Fig. 3a). Hybridization was at 55 °C. Autoradiography was performed at -70 °C for 10 days with an intensifying screen. The size markers used (in kb) were the *Hind*III cleavage products of phage λ DNA⁴³.



by hybridization with probe 2 to differ between the +/+ and the mdg/mdg mouse. For *Apa*I, *Bam*HI and *Kpn*I fragments, however, probe 1 showed no apparent difference between the two mice. Probe 5, which represented a region between those targeted by probes 2 and 6, detected the same *Apa*I fragments that were shown by hybridization with probe 6 to differ between the two mice. However, probe 5 also revealed common restriction fragments in the two mice for all four endonucleases, including *Apa*I. Probes 7, 8, and 9, which represented regions 3' to that targeted by probe 6, detected no difference in restriction patterns between the +/+ and the mdg/mdg mouse. Probes 3 and 4, extending over wide regions of the protein-coding sequence which mostly overlapped the regions targeted by other probes, revealed no apparent alterations in restriction fragments in the mdg/mdg mouse other than those seen in Fig. 1.

We concluded from these results that the differences in restriction fragments between the +/+ and the mdg/mdg mouse observed with probes 2 and 6 were the only differences detected with the nine probes which, taken together, covered the entire protein-coding sequence of the DHP receptor cDNA. Essentially identical results were obtained from genomic DNAs from three +/+, three mdg/mdg and two +/mdg individuals (including the ones shown in Fig. 1). These results indicate that +/+ and mdg/mdg mice show differences in at least two regions of the structural gene for the skeletal muscle DHP receptor.

The muscular dysgenesis mutation arose spontaneously and is transmitted as a single recessive defect. The colony of mice used for our experiments was obtained by transferring the muscular dysgenic defect onto a background of 129/ReJ mice (Jackson Laboratory) by four generations of breeding. Subsequently, the colony has been maintained for 16 years as a small, closed population. Thus, the muscular dysgenic defect is likely to be attributable to a single mutated locus and this mutated locus is present in a highly inbred colony of mice. The association of the defect with alterations of the structural gene for the skeletal muscle DHP receptor suggests that the pathological consequences of muscular dysgenesis are a result of mutation of this gene.

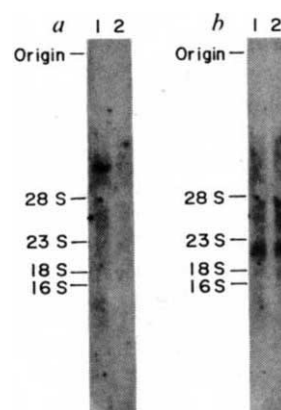


Fig. 2 Autoradiograms of blot hybridization analysis of poly(A)⁺ RNA from skeletal muscle of normal (+/+) and dysgenic (mdg/mdg) mice using cDNA probe for the DHP receptor (a) or the nicotinic acetylcholine receptor β -subunit as a control (b). Lane 1, 18-day-old embryonic +/+; lane 2, 18-day-old embryonic mdg/mdg. Samples of poly(A)⁺ RNA (20 μ g each) prepared^{44,45} from skeletal muscle were analysed as described⁴⁶, except that dextran sulphate was omitted from the hybridization buffer and that salmon sperm DNA was replaced by *Escherichia coli* DNA. The probes used (labelled⁴² with [α -³²P]dCTP; specific activity, $\sim 1 \times 10^9$ c.p.m. μ g⁻¹) were the *Pst*I(111)/*Sac*I(651) fragment (see Fig. 3a) (a) and the *Pvu*II(381)/*Pvu*II(1,279) fragment derived from clone pACR β 110 (ref. 47) (b). Hybridization was at 38 °C. Washing was at 46 °C with 0.3 $\times$ SSC containing 0.1% sodium dodecyl sulphate. Autoradiography was at -70 °C with an intensifying screen for 13 days (a) or seven days (b). The size markers used were mouse and *E. coli* ribosomal RNAs⁴⁸. The other fragments of the DHP receptor cDNA used as probes (see the text) were the *Hind*III(5'-terminal linker)/*Hind*III(3'-terminal linker), *Hind*III(5'-terminal linker)/*Eco*RI(1,007), *Apa*I(804)/*Apa*I(3,098), *Apa*I(3,098)/*Apa*I(4,187) and *Apa*I(4,187)/*Apa*I(5,131) fragments and an equimolar mixture of the three *Apa*I fragments (see Fig. 3a); some experiments were conducted essentially by the procedure described previously⁴⁹.

stimulation. The restoration of E-C coupling was observed anywhere from 2 to 10 days after injection of pCAC6 DNA. As a control experiment, the vector plasmid pKCRH2 (ref. 20), which is identical with the expression plasmid pCAC6 except that it does not contain the DHP receptor cDNA, was injected instead of pCAC6. Of the ~200 dysgenic myotubes that survived injection with pKCRH2, none were observed to contract either spontaneously or in response to electrical stimulation.

Restoration of slow calcium current

Normal skeletal muscle cells acutely isolated from vertebrates show a slowly activated calcium current (I_{slow})^{21,22} which is blocked by DHP (refs 4, 5, 23). The presence of I_{slow} has also been reported for embryonic skeletal muscle cells grown in primary tissue culture^{16,23,24}. Figure 5a illustrates I_{slow} in a normal mouse myotube (I_{slow} is the maintained current that is prominent for test depolarizations $\geq +20$ mV). In addition to I_{slow} , a transient calcium current (I_{fast}) is also present in normal myotubes (Fig. 5a; I_{fast} is prominent for test depolarizations ≤ 0 mV). The presence of these two components of calcium current is evident in the biphasic shape of the peak current-voltage relationship (Fig. 5a, bottom). As described previously^{23,24}, I_{fast} and I_{slow} in rodent myotubes have properties similar to those of the T- and L-type calcium currents, respectively, found in many cell types^{6,7}. Also as described previously^{16,17}, the muscular dysgenesis mutation selectively eliminates I_{slow} without affecting I_{fast} (Fig. 5b).

The fact that the cDNA encoding the sodium channel polypeptide, which shares common structural features with the DHP receptor⁹⁻¹², can be expressed to form the functional channel²⁵⁻²⁷ suggests that the skeletal muscle DHP receptor may be responsible for the I_{slow} calcium current of normal skeletal muscle. If this is the case, injection of the expression plasmid pCAC6 carrying its cDNA into dysgenic myotubes would be expected to restore I_{slow} . To test this prediction, we examined whole-cell calcium currents in injected myotubes that had been seen to contract spontaneously or in response to electrical stimulation. Figure 5c demonstrates that an injected myotube which had been observed to contract spontaneously exhibited not only I_{fast} but also an I_{slow} calcium current with kinetics and voltage dependence similar to those of I_{slow} in normal myotubes. Altogether, we were successful in obtaining whole-cell recordings from 14 injected myotubes that had been observed to contract (four spontaneously and 10 in response to electrical stimulation). All of these 14 cells showed a slow calcium current similar to that illustrated in Fig. 5c; the maximum value of I_{slow} in these cells was 1.1 ± 0.8 nA (mean $\pm$ standard deviation; range 0.4–3.0 nA). In addition, we recorded whole-cell currents from 11 dysgenic myotubes that had not been observed to contract; these cells all possessed I_{fast} but lacked I_{slow} . All of these 11 cells were in areas of the culture where injections had been performed, but we cannot be certain whether all of them were injected or whether they arose from non-injected myoblasts that migrated into the injection areas during the several days after the injection.

To investigate whether I_{slow} in injected cells has properties similar to those of I_{slow} in normal myotubes, we characterized the inactivation and pharmacological properties of the current. For current measurements in both normal and injected myotubes, a standard holding potential of -80 mV was used. In six injected myotubes, briefly (for 1 s) changing to a holding potential of -30 mV or -40 mV inactivated I_{fast} without affecting I_{slow} . In two injected myotubes, prolonged holding at a depolarized potential (> 5 min at -25 mV or -30 mV) inactivated I_{slow} by ~50%. Thus, the effects of holding potential on I_{slow} in injected myotubes are very similar to those in normal myotubes²³. Also similar is the block of I_{slow} by the dihydropyridine (+)PN 200-110, as shown in Fig. 6, which compares the effects of $1 \mu\text{M}$ drug on a normal myotube (a) and an injected myotube (b). We did not characterize the complete

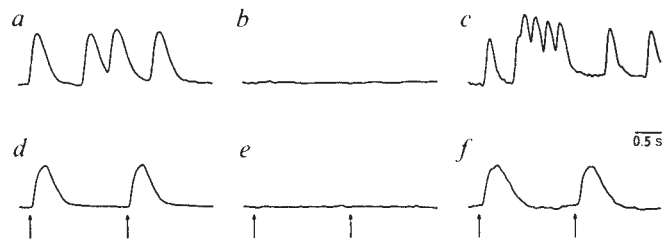


Fig. 4 Comparison of contractile activity in primary cultures of normal (+/mdg?) myotubes (a, d), dysgenic (mdg/mdg) myotubes (b, e) and dysgenic myotubes that were injected with the expression plasmid pCAC6 carrying the DHP receptor cDNA (c, f). The contractile activity in c and f was measured three days after injection with pCAC6 DNA. Spontaneous contractile activity is illustrated in a, b and c; electrically evoked contractile activity is shown in d, e and f. Arrows indicate the time of application of the electrical stimulus. Horizontal calibration: 500 ms. Vertical scale: arbitrary units.

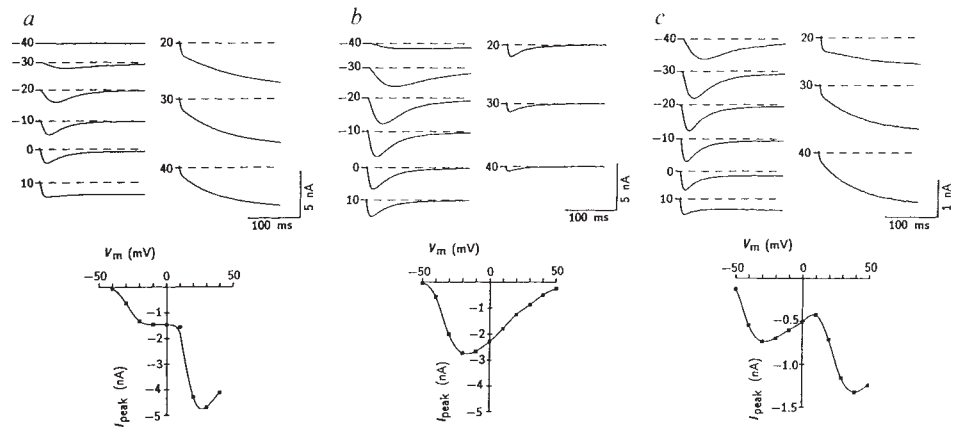
Methods. Primary cultures of myotubes were prepared from newborn mice essentially following the procedure described previously^{16,23}. Cells were plated at a density of 2×10^4 per 35-mm Falcon Primaria dish on which a grid of ~2 mm squares had been ruled. After fusion of myoblasts into myotubes had begun, some of the cultures were treated for 24 h with $10 \mu\text{M}$ cytosine arabinoside. Squares with 10–20 large myotubes ($\sim 15 \times 200 \mu\text{m}$) were selected for injection. Approximately 900 copies of pCAC6 plasmid were injected¹⁹ into each of several nuclei within every myotube inside the square. An Olympus IMT2-SYF 'injectoscope' was used for the injections. Contractions were monitored as a change in the voltage drop across a small photoresistor that was placed at the edge of a myotube's image on a television monitor. The voltage signal from the photoresistor was filtered at 5–10 Hz. For electrical stimulation, pulses were applied through an extracellular pipette placed near the cell. The stimulating pulse lasted for 10 ms and its amplitude was adjusted to be suprathreshold for evoking contraction of normal myotubes.

dose-response curve for (+)PN 200-110, but we measured the reduction of I_{slow} by $1 \mu\text{M}$ drug in five injected myotubes and the reduction by 200 nM drug in one injected myotube. Assuming 1:1 blocking stoichiometry, the half-blocking concentration of (+)PN 200-110 for injected myotubes was $204 \pm 62 \text{ nM}$ ($n = 6$), close to the value of 182 nM found previously²³ for normal rat and mouse muscle under similar conditions.

Because injection of the expression plasmid carrying the DHP receptor cDNA restores I_{slow} in dysgenic myotubes, one might argue that the restoration of depolarization-induced contraction is simply a consequence of Ca^{2+} entering the myoplasm by way of the voltage-gated slow calcium channel. Depolarization-contraction coupling of this sort would differ from E-C coupling of normal vertebrate skeletal muscle, which does not require the entry of extracellular Ca^{2+} (refs 28–30). To test whether entry of external Ca^{2+} is involved in the depolarization-contraction coupling of injected myotubes, we examined the effect of equimolar substitution of Mg^{2+} for Ca^{2+} in the myotube bathing medium. Even after 10 min in the Ca^{2+} -free medium, electrical stimulation caused contraction of both normal and injected myotubes, although an individual cell usually contracted only 5–10 times, after which it became refractory to further stimulation. This refractoriness may reflect a dependence of E-C coupling on extracellular Ca^{2+} (refs 30, 31). Therefore, we also tested the effects of adding 0.5 mM Cd^{2+} to the Ca^{2+} -containing culture medium. This concentration of Cd^{2+} was sufficient to block both I_{fast} and I_{slow} in normal²³ and injected myotubes. After a 10-min exposure to Cd^{2+} , both normal and injected myotubes contracted repeatedly in response to electrical stimulation. These observations indicate that injection of the expression plasmid carrying the DHP receptor cDNA into dysgenic muscle restores E-C coupling to that of normal muscle in which T-tubular depolarization causes the SR to release Ca^{2+} .

Fig. 5 Comparison of calcium currents in a normal (+/*mdg*?) myotube (*a*), a dysgenic (*mdg/mdg*) myotube (*b*) and a dysgenic myotube that was injected with the expression plasmid pCAC6 (*c*). Calcium currents were measured using the whole-cell variant of the patch-clamp technique^{23,51}. The test potential (in mV) is indicated next to each current trace. The peak current (I_{peak}) is plotted against test potential (V_m) beneath the current traces for each cell. Holding potential was -80 mV.

Methods. Calcium currents were corrected for linear components of capacitive and leak currents by digital scaling and subtraction of a control current elicited by a 20 mV hyperpolarization from the holding potential. This control current was also used to calculate the linear capacitance (C) for each cell. *a*, Cell H87, $C = 270$ pF; *b*, cell I28, $C = 620$ pF; *c*, cell I34, $C = 240$ pF. The bathing solution contained (in mM): 10 Ca^{2+} , 145 tetraethylammonium ion, 165 Cl^- , 10 HEPES (pH 7.4 with CsOH). Tetrodotoxin (5–10 μM) was present to block sodium currents. The pipette solution contained (in mM): 140 Cs-aspartate, 5 MgCl_2 , 10 Cs_2EGTA , 10 HEPES (pH 7.4 with CsOH).



Discussion

These results indicate that the muscular dysgenesis mutation alters the structural gene for the skeletal muscle DHP receptor, with the following consequences in skeletal muscle: the level of the mRNA encoding this receptor is strongly reduced, E-C coupling fails, and I_{slow} is absent. Previously, it has been shown that alterations of the structural gene for the A-type potassium channel are responsible for the *Shaker* gene mutation of *Drosophila melanogaster*³². Muscular dysgenesis now seems to provide the first documented example of a genetic disease of a vertebrate that is caused by a defect in a structural gene for an ionic channel.

The I_{slow} calcium current of skeletal muscle has many similarities to the slow L-type calcium currents of other tissues^{6,7}. Because normal L-type currents are present in cardiac cells and sensory neurons of dysgenic mice¹⁶, the structural gene(s) for L-type calcium channels in these cells must be distinct from the structural gene for the skeletal muscle DHP receptor. Consistent with this conclusion is a study of binding of radiolabelled DHP, which indicates that the muscular dysgenesis mutation causes a fivefold decrease in DHP-binding sites in skeletal muscle, but does not affect the DHP binding in heart³³. It is possible that the residual DHP binding in dysgenic skeletal muscle may be due to the RNA that hybridizes to the DHP receptor cDNA, observed in small amounts. However, monoclonal antibodies fail to detect the DHP receptor protein in dysgenic skeletal muscle³⁴, and dysgenic skeletal muscle lacks E-C coupling and slow calcium current. These results may indicate that the RNA observed in reduced amounts in dysgenic skeletal muscle does not encode a fully functional DHP receptor. Another possibility

is that the low amount of this RNA may derive from coexisting vascular tissue.

Our conclusion that the muscular dysgenesis mutation alters the structural gene for the skeletal muscle DHP receptor is at odds with a recent report¹⁷, which claimed that the disease is unlikely to be a result of mutation of the Ca^{2+} channel protein or a structural component of triadic junctions. This argument is based on the observation that co-culture of dysgenic myotubes with spinal cord cells from normal animals restores contraction^{17,35}, normal muscle ultrastructure^{17,36} and I_{slow} (ref. 17), whereas co-culture with dysgenic spinal cord cells is ineffective. On the basis of these results, it was suggested that a nerve-muscle interaction that is important for muscle development is defective in dysgenic mice¹⁷. Because the spinal cord contains both neuronal and non-neuronal cells, an alternative explanation is that non-neuronal cells are responsible for the 'rescue' of dysgenic myotubes by spinal cord co-culture. Pure populations of normal fibroblasts have recently been shown to be effective in rescuing E-C coupling in dysgenic myotubes³⁷. In addition, fibroblasts have been shown to spontaneously fuse with myotubes at low frequency³⁸. If, after fusion with a myotube, a normal fibroblast nucleus begins to express skeletal muscle-specific proteins³⁹ including the DHP receptor, this would explain the observed rescue. Thus, the rescue of dysgenic muscle by normal spinal cord cells does not necessarily imply that the skeletal muscle DHP receptor gene is intact in the *mdg/mdg* genome.

That both E-C coupling and slow calcium current missing from dysgenic muscle can be restored by microinjection of the expression plasmid carrying the skeletal muscle DHP receptor cDNA strongly supports the view that this receptor molecule in the T-tubule membrane has a dual function⁹. It is an essential component of E-C coupling, probably acting as the voltage sensor, and also functions as the slow calcium channel.

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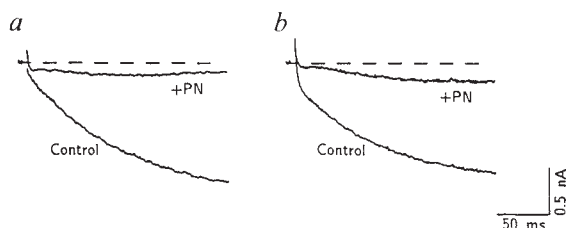


Fig. 6 DHP block of I_{slow} in a normal (+/*mdg*?) myotube (*a*) and in a dysgenic (*mdg/mdg*) myotube that was injected with the expression plasmid pCAC6 (*b*). I_{slow} was measured before (control) and during exposure to 1 μM (+)PN 200–110. *a*, Cell I49, test pulse = +40 mV, $C = 150$ pF; *b*, Cell I34, test pulse = +30 mV, $C = 240$ pF. Additional experimental details are given in Fig. 5 legend.

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Histidine phosphorylation and phosphoryl group transfer in bacterial chemotaxis

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A cascade of protein phosphorylation, initiated by autophosphorylation of the CheA protein, may be important in the signal transduction pathway of bacterial chemotaxis. A proteolytic fragment of CheA cannot autophosphorylate, but can still transfer phosphate to proteins that generate excitation and adaptation signals. The site of CheA phosphorylation is His 48; mutants altered at this position are non-chemotactic. Similar mechanisms of transient protein phosphorylation and phosphoryl group transfer seem to be involved in processing sensory data and in activating specific gene expression.

THE regulation by transient phosphorylation of the activity of a protein is a common feature in a wide variety of biological processes, including signal transduction, growth control and metabolism. In eukaryotes, the protein kinases that mediate protein phosphorylation can be grouped into families of functionally related members on the basis of the amino-acid residue they phosphorylate and by similarities between catalytic-domain amino-acid sequences¹. In prokaryotes, protein phosphorylation was recently discovered in a family of proteins involved in signal transduction and regulation of gene expression^{2,3}. A more complete understanding of this prokaryotic protein kinase family, and of possible functional, mechanistic, or evolutionary relationships with its eukaryotic counterparts, requires the identification of the catalytic regions and of the phosphorylated amino acids.

Biochemical analyses using purified chemotaxis proteins from *Escherichia coli* suggest that protein phosphorylation is important in sensory transduction in the chemotaxis system³⁻⁶. A model of chemotaxis incorporating *in vitro* biochemical data and previous genetic results⁷⁻¹⁰ is shown in Fig. 1. Chemotaxis results from modulation of the direction of rotation of flagellar filaments in response to extracellular stimuli^{11,12}. Sensory information gathered by a set of transmembrane receptors is integrated and transmitted to the flagellar 'switch' (that is, the component of the flagellar motor that regulates rotation) by four cytoplasmic proteins, CheA, CheW, CheY and CheZ^{13,14}. Adaptation to environmental stimuli (that is, feedback regulation of receptor function) correlates with specific methylation

and demethylation of the receptors by the cytoplasmic proteins CheR and CheB¹⁵⁻¹⁷. CheA is an autophosphorylating protein kinase that can transfer phosphate to the CheB and CheY proteins³⁻⁵. Phosphorylation of CheY presumably generates a cellular response, whereas phosphorylation of CheB serves in a negative feedback loop by mediating adaptation of the transmembrane receptor proteins. CheB and CheY are regenerated by dephosphorylation⁴⁻⁶. Therefore, chemotaxis provides an opportunity to investigate a complete circuit of information processing involving protein phosphorylation.

Here we describe studies of the structure and function of the CheA protein. The N-terminal quarter of the protein seems to be a phosphorylated domain which is competent to transfer the phosphoryl group to CheB or CheY. The site of phosphorylation in the CheA protein is His 48. The effects of mutations at the phosphorylation site and at an adjacent serine residue have been examined. The results provide evidence in support of the proposed role of phosphorylation in bacterial chemotaxis and indicate that phosphohistidine is important in signal transduction systems.

CheA phosphotransferase domain

Partial proteolytic digestion was used to explore the domain structure of CheA. Digestion of purified [³²P]CheA-phosphate with trypsin produced a number of polypeptide fragments that were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 2a). Phosphate was associated with a peptide fragment of relative molecular mass of about 18,000 (18K) that was present in relatively constant amounts throughout the time

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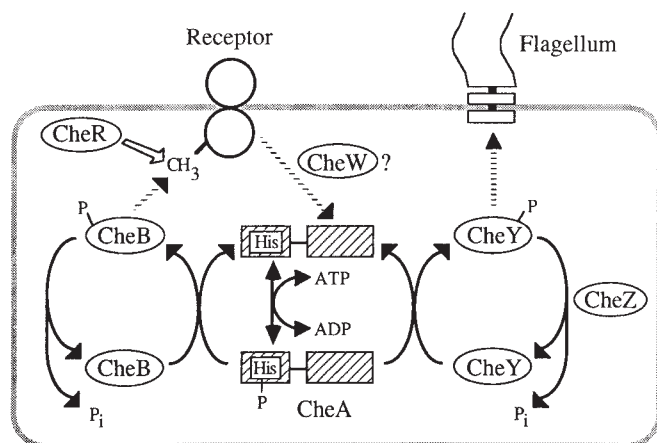


Fig. 1 Model of signal transduction in chemotaxis by *E. coli*. Information about the external environment is detected and transmitted across the cell membrane by the receptor/transducer proteins. The transducers affect CheA in an as yet unknown manner, possibly involving CheW, to modulate phosphorylation of CheA. The site of phosphorylation is His 48 within an N-terminal phosphotransferase domain of CheA. Phosphate is transferred from CheA to CheB and CheY to generate adaptation and excitation signals respectively. Phosphorylation of the CheB protein has been proposed to enhance its methyltransferase activity. The CheR methyltransferase activity counteracts CheB (outlined arrow). CheY-phosphate is proposed to act at the flagellar switch to alter swimming behaviour, but can be dephosphorylated (inactivated) by CheZ. The phosphate transfer reactions indicated by solid arrows have been shown to occur *in vitro*³⁻⁶, whereas the interactions indicated by dashed arrows are hypothesized to occur *in vivo*.

course and after 30 min of digestion was the largest main band remaining. The relative resistance of this 18K fragment to tryptic digestion indicated that it represented a structural domain of CheA. The fragment was isolated and purified by reverse-phase high-performance liquid chromatography (HPLC). The sequences of the N-terminal 14 amino acids of the fragment and of the intact CheA protein were identical. Thus, the 18K fragment is the N-terminal domain of the CheA protein.

To investigate the activity of this domain, intact CheA was autophosphorylated, extensively digested with trypsin, and the 18K fragment purified using a fast protein liquid chromatography (FPLC) gel filtration column. Incubation of the purified phosphorylated N-terminal domain of CheA with CheB or CheY in the presence of MgCl₂ resulted in the rapid dephosphorylation of the fragment and the transient phosphorylation of CheY (Fig. 2b) or CheB (Fig. 2c). The relative rates of these reactions were similar to those previously observed for full-length CheA-phosphate⁴; thus, the N-terminal domain of CheA retained the phosphotransferase activities of the full-length protein. This localization of transferase activity is consistent with previous observations: mutations that reduce phosphate transfer from CheA to CheB and CheY map to the N-terminal region of CheA¹⁸. CheA-phosphate can transfer phosphate to ADP (ref. 3), but the phosphorylated N-terminal domain did not (data not shown). The 18K fragment prepared from unphosphorylated CheA was not capable of autophosphorylation (data not shown), which is consistent with genetic analysis suggesting that other regions of CheA are necessary for autophosphorylation activity¹⁸.

Identification of the phosphorylation site

The region of phosphorylation within the N-terminal domain of CheA was determined by sequential proteolytic digestion of ³²P-labelled CheA-phosphate and isolation of phosphorylated peptides by reverse-phase HPLC. After digestion of intact CheA-phosphate with trypsin to generate the N-terminal domain, radioactivity was associated with three peaks (1, 2 and 3) (Fig.

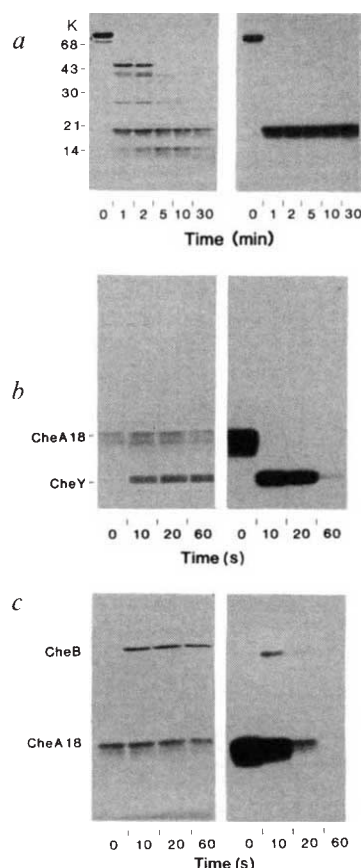


Fig. 2 Identification (a) and activity (b, c) of a phosphorylated proteolytic fragment of [³²P]CheA-phosphate. Digestions or reactions were stopped at the times indicated by the addition of SDS-sample buffer³, and the products analysed by SDS-PAGE. In each panel, the left half shows a Coomassie blue stain and the right half an autoradiogram of the gel. The positions of relative molecular mass standards are indicated on the left. A phosphorylated 16K fragment was detected in addition to the 18K fragment (Fig. 1b, c). The 16K fragment seems to be the result of further digestion of the C-terminus of the 18K fragment, because the 16K fragment seemed to have an N-terminal amino-acid sequence identical to that of the 18K fragment (data not shown).

Methods. a, Purified phosphorylated CheA was partially digested with trypsin at a w/w ratio of 100 CheA:1 L-1-tosylamide-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin (Sigma). b, c, CheA-phosphate was digested for 90 min with trypsin (w/w ratio of 20 CheA:1 trypsin), soybean trypsin inhibitor added (w/w ratio of 2 inhibitor:1 trypsin), and the fragment purified on a Pharmacia Superose-12 FPLC column. Phosphate transfer reactions were performed as previously described⁴, with the phosphorylated CheA fragment present in a twofold molar excess with respect to CheY (b) and a fourfold molar excess with respect to CheB (c).

3a). Amino-acid sequence analysis of pooled peaks 1, 2 and 3 indicated that they contained peptides with N termini that corresponded to the N terminus of the intact CheA molecule. Peaks 1, 2 and 3 were pooled, digested with chymotrypsin and the products separated as before (Fig. 3b). Radioactivity was associated with a single new peak (4). The N-terminal sequence of the major peptide in peak 4 began with Arg 45 and contained at least ten amino acids. A second, minor peptide with an overlapping sequence was also present in this peak. Peak 4 was digested with a mixture of chymotrypsin and trypsin and the products separated (Fig. 3c). Radioactivity was associated with major (5) and minor (6) peaks. N-terminal amino-acid sequence analysis showed that peak 5 consisted of one peptide beginning with Arg 45 with the sequence Arg-Ala-Ala-His-Ser-Ile-Lys (RAHSIK) and peak 6 consisted of a single peptide beginning

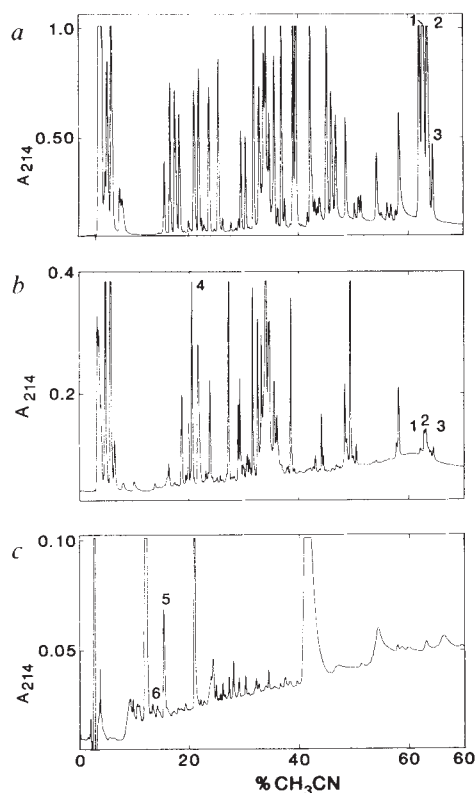


Fig. 3 Isolation of phosphorylated CheA peptides by reverse-phase HPLC. Peptides were eluted with a 0–60% linear gradient of acetonitrile in water containing 0.1% trifluoroacetic acid. The gradients were developed over 60 min at a flow rate of 0.8 ml min⁻¹ and maintained at 60% acetonitrile for 10 min. Absorbance at 214 nm is shown. Peaks containing radiolabelled peptides are designated 1–6. *a*, Purified [³²P]CheA (500 µg) was digested with trypsin (25 µg) for 90 min. The reaction was applied to a Vydac C-4 HPLC column and eluted. Approximately 55% of the applied radioactivity was recovered: 45% in peaks 1, 2 and 3, and 10% as inorganic phosphate (P_i), which did not interact with the column. *b*, Peaks 1, 2 and 3 were pooled, lyophilized, resuspended in TEDG buffer (ref. 4; 50 mM Tris HCl pH 7.5, 0.5 mM EDTA, 2 mM dithiothreitol, 10% glycerol) digested with 8 µg chymotrypsin for 2 h, applied to the same C-4 column, and eluted. Approximately 85% of the applied radioactivity was recovered: 20% as P_i, 55% in peak 4, and 10% was undigested. *c*, Peak 4 was lyophilized, resuspended in TEDG buffer, digested with 2 µg chymotrypsin and 2 µg trypsin for 90 min, applied to an Applied Biosystems C-18 HPLC column, and eluted. Approximately 80% of the radioactivity was recovered: 10% as P_i, 5% in peak 6, and 65% in peak 5.

with Ala 46 with the sequence AAHSIK. Peak 5 was the predominant radioactive peptide, and presumably resulted from the inability of trypsin to cleave arginine residues efficiently at the N terminus of a peptide¹⁹. The only amino acids shared by peptides 5 and 6 which could be phosphorylated are His, Ser and Lys. Given the substrate specificity of trypsin²⁰, which was used to generate the peptides, it seems highly unlikely that the lysine residue was phosphorylated. Therefore, His 48 and Ser 49 were considered to be the best candidates for the site of phosphorylation in CheA.

To identify the phosphorylated residue, we tried to degrade peptide 5 to individual amino acids. Because treatment with strong acid destroys phosphohistidine and treatment with strong base destroys phosphoserine²¹, proteolytic digestion was used to cleave the peptide bonds under conditions of neutral pH. The phosphorylated peptide RAAHSIK was isolated as described above and digested with a mixture of carboxypeptidases B and Y. The products were analysed by two-

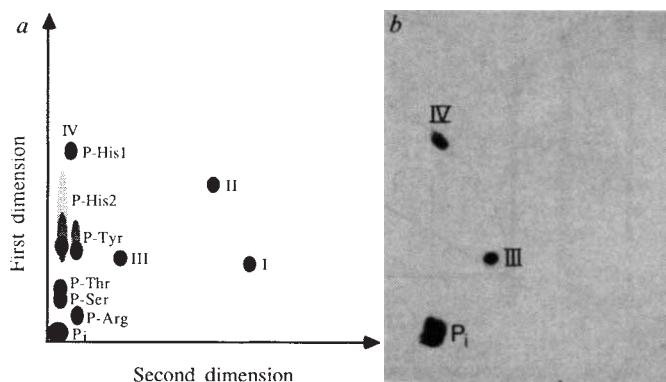


Fig. 4 Two-dimensional TLC of products of digestion of ³²P-labelled peptide 5 (Fig. 3c) with carboxypeptidases B and Y. *a*, Composite representation from multiple TLC plates identifying standards visible by ninhydrin staining (stippled) and digestion products (I–IV) visible by autoradiography (solid). *b*, Autoradiogram of two-dimensional TLC of a 14 h digestion of 3 pmol [³²P]RAAHSIK (500 c.p.m. pmol⁻¹) with 0.1 units carboxypeptidase Y and 0.5 units carboxypeptidase B.

Methods. Chromatography on Silica Gel 60 K6 (Whatman) was with chloroform:methanol:17% w/w ammonia (2:2:1, v/v) in the first dimension, and with phenol:water (75:25, w/w) in the second dimension⁴⁶. Phosphoamino acids (phosphoarginine, phosphoserine, phosphothreonine and phosphotyrosine) were purchased from Sigma. The phosphohistidine standard was prepared by mixing 100 mg potassium phosphoramidate⁴⁷, with 10 mg histidine in 1 ml of water at room temperature for 2 h and storing at –20 °C. Under these conditions the N-1, the N-3, or both positions of the imidazole ring of histidine can be phosphorylated⁴⁸, as can the α-nitrogen⁴⁹. The phosphohistidine standard was further characterized by examining the time-course of histidine phosphorylation^{48,49}. Two distinct species designated P-His1 and P-His2 were observed. P-His1 was produced rapidly (within 15 min) and remained relatively constant over 2 h, whereas P-His2 accumulated with time. In addition, the elution profile of the phosphohistidine species from a Dowex AG1-X8 column⁵⁰ was examined. P-His1 eluted from the Dowex column just before P-His2. These characteristics indicate that p-His1 corresponds to a monophosphohistidine, possibly 1-phosphohistidine.

dimensional TLC (Fig. 4). Before treatment with proteases, the peptide migrated at position I (Fig. 4a), with some label also present as inorganic phosphate. Partial digestion with the carboxypeptidases resulted in the production of two additional radioactive spots, designated II and III, which seemed to be susceptible to further digestion by proteases (data not shown) and did not co-migrate with any of the phosphoamino acids tested. Spots II and III seem to be labelled peptides generated by the sequential removal of amino acids from the C-terminus of RAAHSIK. Extended digestion of RAAHSIK with the carboxypeptidase mixture resulted in the production of spot IV, as well as a new radioactive spot (IV) (Fig. 4b). Spot IV co-migrated with a spot, P-His1, in the phosphohistidine standard. The mobility characteristics of spot IV are consistent with its being a monophosphorylated form of histidine, possibly 1-phosphohistidine (see Fig. 4 legend). We therefore conclude that the site of phosphorylation in CheA is His 48. Previous observations of the lability of CheA-phosphate to treatment with acid, base, hydroxylamine, or pyridine are consistent with phosphohistidine^{3,5}.

Mutations at the phosphorylation site

If phosphorylation is important in signal transduction in bacterial chemotaxis, we would expect the site of phosphorylation in CheA to be essential for chemotaxis. To test this, site-specific mutagenesis²² was used to change His 48 to 13 different amino acids: Ala, Arg, Asp, Glu, Gln, Gly, Ile, Leu, Lys, Pro, Ser, Thr,

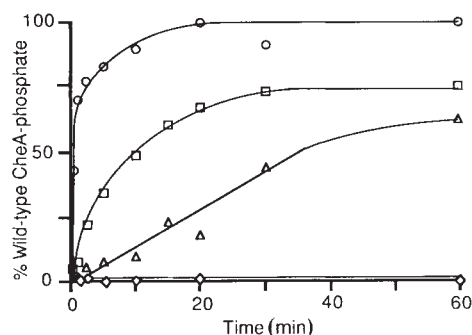


Fig. 5 Time course of autophosphorylation of wild-type and mutant CheA proteins. Proteins were: wild-type, (○); 49ST, (□); 49SA, (△); and 48HQ (◇).

Methods. Purified proteins were incubated with 5 mM MgCl₂ and 50 mM KCl in TEDG buffer⁴. Reactions were initiated by the addition of 0.5 mM [γ -³²P]ATP (5,000 c.p.m. mol⁻¹). Aliquots were removed at the indicated times, spotted on to glass fibre disks, and precipitated with trichloroacetic acid³. The amount of phosphorylation at each time point is expressed relative to the amount of CheA-phosphate (~10 pmol) at the endpoint of the reaction with wild-type CheA. Combined results of two experiments are shown.

or Val. Each mutation was placed in the *cheA* gene of plasmid pDV4 (ref. 23) and transformed into KO685 (ref. 18), a strain of *E. coli* deleted for *cheA*. None of the 13 mutations was able to complement CheA function, that is, chemotaxis was not observed. The swarm phenotype of each of these mutants was indistinguishable from the parent *cheA* deletion strain, either without a plasmid or bearing plasmids with nonsense mutations at codon 48. Therefore, it seems that the site of phosphorylation in CheA, His 48, is essential for chemotaxis.

Because Ser 49 was also a candidate for the site of phosphorylation, we changed this residue to either Ala or Thr. Both mutations resulted in strains that were clearly defective in chemotaxis. The Ser to Ala mutant showed no apparent chemotaxis, but some residual chemotaxis was observed on swarm plates with the Ser to Thr mutant. These results indicate that Ser 49 is also important in CheA function.

To examine further the role of His 48 and Ser 49 in CheA function we purified three mutant proteins, containing alterations of either His 48 to Gln (48HQ), Ser 49 to Ala (49SA), or Ser 49 to Thr (49ST), and tested their *in vitro* autophosphorylation activity (Fig. 5). Even after long reaction times, the mutant protein 48HQ was not phosphorylated, consistent with the conclusion that His 48 is the site of autophosphorylation. In contrast, the mutant proteins 49SA and 49ST were phosphorylated, although at rates significantly lower than wild-type CheA. With long reaction times, the level of phosphorylation of the 49SA and 49ST mutant proteins approached that of wild-type CheA. These results indicate that Ser 49 is important in the mechanism of autophosphorylation, but provide evidence against the possibility that Ser 49 serves as a site of phosphorylation. The 49ST mutant protein, which seemed to support some residual chemotaxis, also had the highest rate of autophosphorylation activity of these mutants. Taken together, these results strengthen the correlation between protein phosphorylation and bacterial chemotaxis.

To test the modified CheA proteins for CheY kinase activity, phosphorylation reactions with mutant or wild-type CheA in the presence of CheY were analysed on SDS-PAGE (Fig. 6). The mutant protein 48HQ did not phosphorylate CheY, supporting the hypothesis that the autophosphorylation of CheA is required for the phosphorylation of CheY. The mutant proteins 49SA and 49ST were phosphorylated and transferred phosphate to CheY, but substantially less CheY-phosphate was formed than by wild-type CheA, presumably as a result of the slow rate of mutant CheA autophosphorylation.

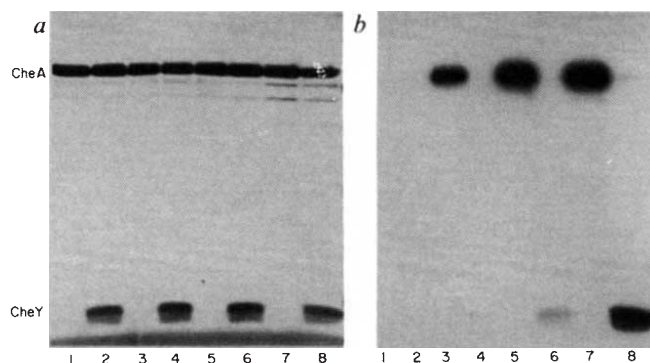


Fig. 6 Autophosphorylation of CheA, and phosphate transfer to CheY, by the 48HQ, 49SA and 49ST mutant CheA proteins, analysed by SDS-PAGE. Coomassie blue staining (a) and autoradiogram (b) of the gel are shown. Lanes: 1-2, 48HQ; 3-4, 49SA; 5-6, 49ST; and 7-8, wild-type CheA.

Methods. Phosphorylation reactions containing 4 μ g CheA were incubated as described in the Fig. 5 legend for 10 min, stopped by the addition of SDS sample buffer³, and analysed by SDS-PAGE. CheY (6 μ g) was present in the reactions analysed in lanes 2, 4, 6 and 8.

Discussion

The CheA protein contains an N-terminal domain that is competent in phosphoryl group transfer to CheB and CheY but does not seem to be sufficient for interactions with ATP/ADP. This N-terminal domain contains the site of CheA autophosphorylation, His 48. Mutagenesis of His 48 indicates that this residue is essential to CheA function in chemotaxis, strengthening the correlation between protein phosphorylation and bacterial chemotaxis. It is interesting that CheA contains the consensus sequence Gly-X-Gly-X-X-Gly, which has been identified in other nucleotide-binding proteins²⁴, immediately to the C-terminal side of the autophosphorylation site²⁵.

Known cases of histidine phosphorylation are less numerous than for hydroxy amino-acid residues (serine, threonine, tyrosine), perhaps partly because of the widespread use of experimental techniques that fail to preserve acid-labile phosphoamino acids. Phosphohistidine-containing intermediates have been described for several metabolic enzymes in bacteria, plants, and mammals²⁶⁻²⁸. A histidine residue has been implicated in the catalytic activity of several eukaryotic serine/threonine protein kinases; however, it is not known whether this residue becomes phosphorylated²⁹. Phosphorylation of histidine has been postulated to regulate the activity of several enzymes³⁰⁻³². In addition, histidine phosphorylation in histone H4 prepared from several different biological sources has been reported^{33,34}. Therefore, histidine phosphorylation potentially has a wide variety of roles in both prokaryotic and eukaryotic organisms.

Phosphohistidine enzyme intermediates are used in the bacterial phosphoenolpyruvate-dependent carbohydrate: phosphotransferase system (PTS) (reviewed in refs 35 and 36). The PTS involves a series of phosphohistidine-mediated phosphoryl group transfer reactions, initiated with phosphoenolpyruvate and ending with sugar phosphorylation and transport. The PTS also influences the chemotactic behaviour of the cell³⁷. This apparently involves the phosphorylation/dephosphorylation of the PTS proteins and requires the participation of *che* gene products³⁸. The PTS could conceivably influence chemotactic behaviour by direct phosphate transfer to CheA, CheB or CheY; phosphoryl group transfer from a PTS enzyme to a non-PTS protein has been observed³¹. The phosphoamino acid(s) in CheB or CheY are not identified; however, the DNA sequence of *cheY* indicates that this protein lacks histidine³⁹.

A family of two-component regulatory systems of prokaryotes governs the response to a variety of environmental stimuli^{25,40-43}.

The best characterized of these systems involves the transcriptional regulation of genes encoding enzymes of the nitrogen assimilation pathway and has previously been shown to involve protein phosphorylation². The NtrB (N_RII) protein, which shares sequence similarities with CheA^{40,43}, is autophosphorylated and in turn transfers the phosphoryl group to the NtrC (N_RI) protein⁴⁴, which has sequence similarities with CheB and CheY^{40,41}. NtrB phosphorylation has been shown to involve phosphohistidine, and transfer to NtrC results in the formation of aspartyl phosphate⁴⁵. The striking resemblances between the Che and Ntr systems suggest that information processing in a variety of two-component systems may proceed through similar mechanisms. Furthermore, protein phosphorylation in eukaryotic cells, which is involved in signal transduction and in the

activation of gene transcription, may also use phosphohistidine. Detailed comparisons of the circuitry in various prokaryotic and eukaryotic signal transducing systems will reveal general mechanisms that integrate and process biological information.

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LETTERS TO NATURE

Is the Gordii Dorsum escarpment on Mars an exhumed transcurrent fault?

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Investigators of deformation features on the surface of Mars have generally concluded that fault zones with major horizontal displacements do not occur or have not been preserved¹⁻⁵. Here we present evidence from high-resolution Viking imagery for an array of structures along the Gordii Dorsum escarpment which suggest that it is a left-lateral strike-slip fault zone of lithospheric proportion. The escarpment is one of an array of subparallel ridges^{6,7} preserved in an equatorial region to the west of Tharsis Montes. The region is of transitional character between the youthful ('Amazonian' age) cover of the Amazonia Planitia to the north and older ('Noachian' age) intensely cratered surfaces of the Highlands to the south. Relations between these ridges and the Gordii Dorsum suggest that the ridges are exhumed ancient fault zones. These features imply that Mars, like the Earth today, may have had in its early stages a more dynamic tectonic style of planetary evolution.

Ancient strike-slip faults on Mars could be difficult to identify. Their traces may readily be masked by later cratering, volcanism

or aeolian erosion and redeposition. Also, where strike-slip faults at depth are covered by poorly consolidated strata, deformation at the surface is likely to be distributed over a broad zone of shearing with or without the development of a surface-breaking strike-slip fault^{8,9}. In these situations characteristic secondary deformation structures commonly develop in the cover strata^{8,9}, but such features would not be easily identified on the low- to moderate-resolution imagery that has been the basis of much of the earlier tectonic studies of the surface of Mars.

Here we present evidence for a set of secondary tectonic features along the Gordii Dorsum escarpment, which are believed to be related to a major left-lateral fault in the lithosphere. The escarpment, with 100-400 m of relief (shadow-length estimates), has been interpreted previously¹⁰⁻¹² as a ~500-km-long normal fault with the east-northeastern side displaced downwards with respect to the west-southwestern side, according to Mariner 9 and Viking data with poor (1-km) to moderate (200-m) resolution. Near the end of the Viking Orbiter 1 mission, a series of high-resolution (35 m per pixel) vidicon images were obtained for the southeastern portions of the Amazonia Planitia, and these images provide excellent coverage of the southern 290 km of the Gordii escarpment. These images were spatially filtered, to emphasize surface relief and minimize albedo variations, and were assembled manually into a map. A strip, marginal to the escarpment (0°-8° N, 138°-148° W, orbits 462S-467S), of width ~80 km, was extracted from the overall mosaic map for this structural investigation (Fig. 2).

On the regional scale the Gordii Dorsum forms a boundary between provinces of contrasting geological character. To the

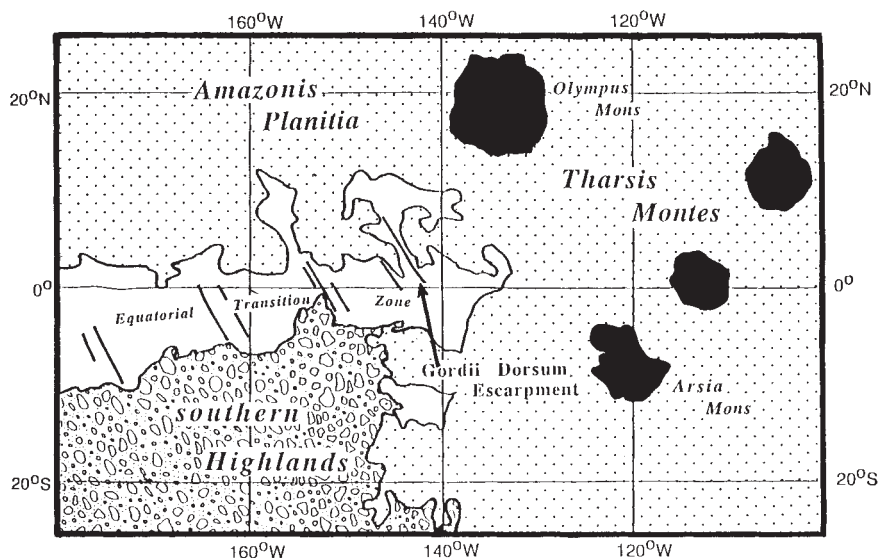


Fig. 1 Location and terrain map for the study area in the equatorial zone of Mars, to the west of the Tharsis Montes. Shown in black are the main volcanic edifices; in 'breccia' pattern, the intensely cratered ancient Noachian surfaces most typical of the highlands of the southern hemisphere, suggested to be intermediate in age (Hesperian) between that of the Highlands areas and of the younger (Amazonian) materials that dominate the northern hemisphere (stippled). Many subdued and linear ridges trend NNW, parallel to the Gordii Dorsum escarpment, but these are largely restricted to the equatorial transition area.

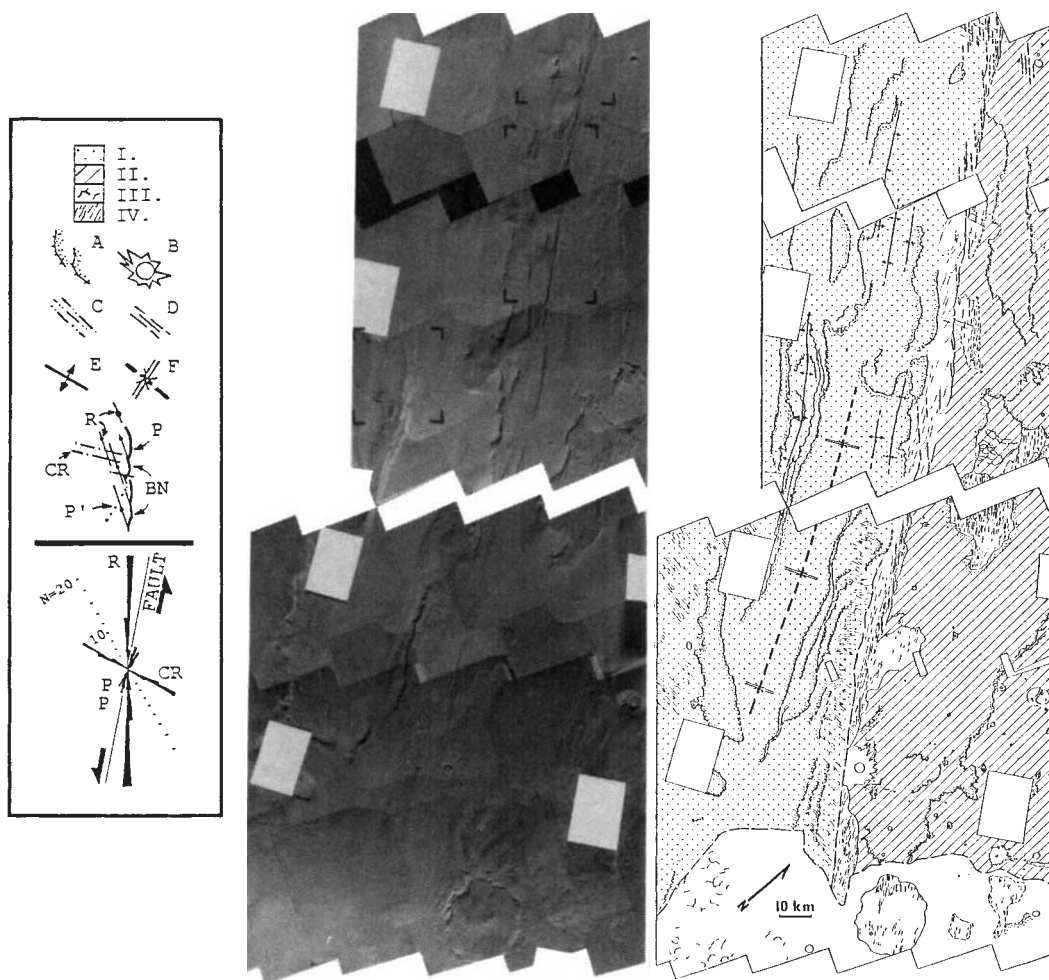


Fig. 2 Viking image mosaic and geological interpretation for a region along the central parts of the Gordii Dorsum escarpment (here interpreted as a left-lateral transcurrent fault). Terrains represent: I, uplifted rolling plains of deeply exhumed, uncratered but deformed strata, probably of Noachian to early Hesperian age; II, sparsely cratered, but undeformed lava (?) plains, probably of Amazonian age; III, moderately exhumed but intensely cratered surface with ridge-and-pinnacle morphology, probably of Noachian age; IV, erosional windows of a highly lineated basement. Symbols are: A, cuestas; B, pedestal craters; C, semi-pervasively linear ground; D, discretely linear ground; E, axial trace of gentle fold; F, general axis of downwarp; R, Reidel shears; CR, conjugate Reidel shears; BN, 'bullnose' structures; P, P-shears; and P', P'-shears (discussed in text).

ENE, along the depression flanking the escarpment, sparsely cratered (51 craters with diameters >0.6 km over $\sim 9,000$ km²), but otherwise undeformed, strata (terrain II, Fig. 2) cover a highly lineated basement terrain (IV) which is visible in erosional windows and in localized basement highs along the base of the escarpment. The cover strata, mapped as Amazonian-age volcanic flow units which were probably erupted from Arsia Mons^{10,12}, exhibit highly irregular cuestas, extensive regions of mottled ground, and pedestal craters. The escarpment appears to have acted as a structural dam to these flows.

To the WSW a thicker sequence of cover (terrain I) has been uplifted (relative to ENE side) and exposed along a structurally controlled NNW-trending series of cuestas. In contrast to the northeastern side, over the 16,000 km² of terrain I there is a conspicuous lack of craters, and exposures of the deeper stratigraphic levels exhibit an east-west, rather than NNW, linear grain. Here youthful exhumation of an older surface appears to be the only mechanism that can adequately explain how the deformed, but uncratered, surface deposits of terrain I are embayed by undeformed, but cratered, units seen at the base

of the escarpment in terrain II.

To the SSE a third region has surface materials that overlap the Gordii Dorsum escarpment without any signs of deformation. Here, the subdued ridge-and-pinnacle topography is suggestive of an ablated, but previously intensely cratered, surface^{13,14}. This region is transitional to terrain I, with the gradual appearance of a structurally controlled cuesta morphology and the disappearance of the ridge-and-pinnacle morphology. Terrain III also seems to be embayed by the cover units of terrain II. These relations imply that the cratered surface of terrain III developed after faulting but before exhumation of terrain I.

Within a 40–50-km fringe on the upwardly displaced side of the escarpment, cuestas and undulations of the surface of terrain I constitute records of gentle folding and/or faulting along subparallel trends to the escarpment. In Fig. 3a there appears to be a gentle, doubly-plunging anticline where the axial culmination is breached by aeolian erosion, and deeper, but exposed, layers show gradual shifts in albedo as they gradually change dip over the fold structure. Elsewhere, there are local undulations which appear to be more step-like.

In the 15–20-km fringes of the escarpment there are four sets of linear features, which are comparable with the arrays of staggered synthetic and antithetic shears that are found along major left-lateral fault zones on Earth and in laboratory clay-drape shear experiments^{8,9}. Three of these are known as Reidel, conjugate Reidel and P-shears. The angular relations (rose diagram in lower inset of Fig. 2) for the main Reidel shears (that is, arrays of staggered left-lateral microfaults) are the same as those seen in laboratory left-lateral shear experiments. Lineaments that probably correspond to the conjugate Reidel (arrays of staggered right-lateral microfault) shears can be discerned only in the NNW portion (Fig. 3a), where the angle made with the fault zone (70–80°) is somewhat lower than those found in experiments, but is not atypical of what has been found in real geological systems⁸. The escarpment itself is formed of a series of en echelon (staggered) north-south trending segments bounded to the NNW and SSE by prominent Reidel shears (Figs 3a and b). Each of these segments forms a conspicuous bulge along the escarpment and gives it a scalloped appearance in map view. The angular and spatial relations of these segments are the same as that described for the P-shears in the experiments. P-shears are the terminations of prominent Reidel shears that accommodate components of reverse faulting in the progressive stages of shearing during which a through-going fault zone is formed. The result is an en echelon series of bulges or push-up structures, called 'bull-nose' structures in reports on clay-drape experiments⁸ and 'positive flower' structures in the petroleum industry^{15,16}. A fourth linear structure, here labelled the P'-shear (subparallel to the P-shear), has not been documented in previous clay-drape shear laboratory experiments. We have performed similar experiments, however, using soft (>40% H₂O) and thin (<1.5-cm) clay layers, in which, following P-shear development, internal shortening of the 'push-up' was accommodated in the late stages by the development of high-angle synthetic shears (P'-shears) which essentially imbricate the push-up in plan view (Fig. 3c).

In the laboratory experiments⁸, resistance to shear across the fault zone peaks during the development of the maximum width of the zone affected by the Reidel shears, and then is quickly reduced, with continued shearing, as the deformation collapses by P-shear formation into a zone more restricted to the trace of the basement fault⁸. In these experiments cumulative displacements up to the development of P-shears usually involves amounts equal to, or greater than, the width of the zone affected by the Reidel shears. The complete array of features seen along the Gordii Dorsum escarpment indicate that this fault zone has evolved to post-peak shear stress stages. Thus, by analogy, we can argue crudely for a minimum left-lateral displacement roughly equal to the width of the zone affected by the Reidel

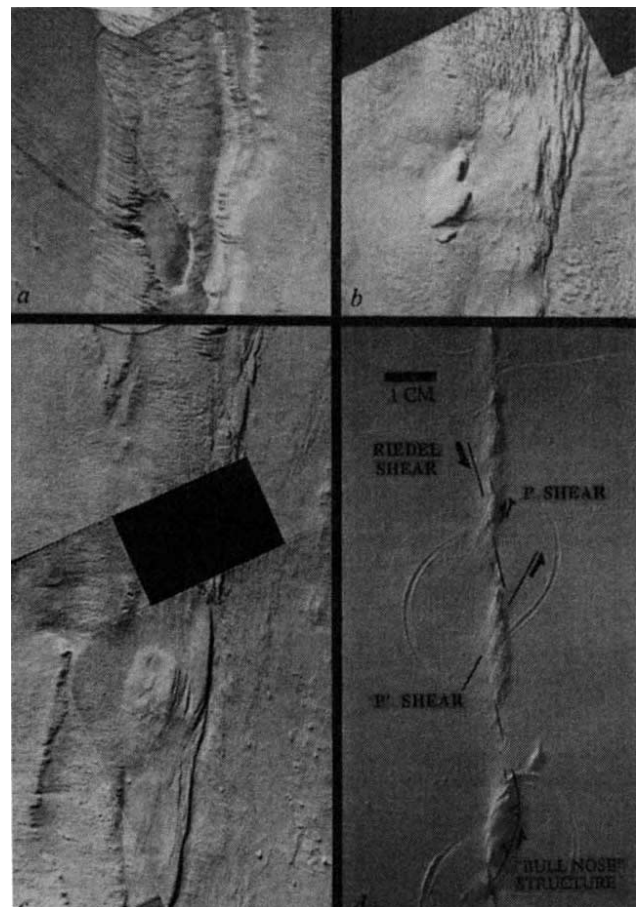


Fig. 3 *a*, Close-up of region of anticlinal folding, showing differential aeolian erosion along axial culmination and gradual albedo shifts on the folded layers. *b*, Close-up image of the north-northwesternmost part of the escarpment, where two prominent sets of highly linear surface features are preserved along the upwardly displaced side. These have angular relations (illustrated in Fig. 2), interpreted as the same as the Reidel and conjugate Reidel shears for left-lateral faults. *c*, Close-up of region of en echelon 'push-up' structures along the Gordii Dorsum escarpment; each appears localized by prominent NNW-trending linear features here interpreted as the primary Reidel shears along a left-lateral fault. *d*, Clay-drape experiment mimicking three of four synthetic shears on the Gordii Dorsum fault zone, and the push-up or 'bullnose' structures along the southwestern upthrown side.

shears. These are exhumed only in the 15–20-km fringe on the upwardly displaced side. Because the downwardly displaced block appears to be buried by younger cover deposits, we are forced to assume a symmetric relationship and to infer a minimum left-lateral displacement of about 30–40 km, which is one to two orders of magnitude greater than the minimum dip-slip motion.

Based on relations seen between terrains I, II and III, the faulting probably occurred before cratering of terrain III. Terrain III has been mapped previously as comprising Hesperian-age surface materials typical of the western equatorial transition zone^{2,10–12}. We argue here, however, that ablation of the surface is quite extensive, and that the surface of terrain III appears to grade gradually into the exhumed uncratered surface of terrain I. Thus it seems most probable that the local crater densities are not so much indicative of the age of surface units but more probably of minimum ages for surface modification. The most logical alternative is that the surface of terrain III is representative of upper Hesperian (or younger) modifications of a lower Hesperian (or older) cratered surface. We suggest that this cratering postdates the faulting, which implies that the faulting is very ancient, probably of Noachian or early Hesperian age.

Although the data presented here for the Gordii Dorsum escarpment do not invalidate the evidence for lithospheric immobility in the greater part of the geological history of Mars, they do suggest that an earlier phase of lithospheric mobility has not been completely obliterated.

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A connection between the X-ray spectral branches and the radio brightness in GX17+2

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GX17+2 (4U 1813-14) is a bright X-ray binary^{1,2} in which matter is accreting on to a neutron star from a nearby companion. X-ray bursts, which are due to thermonuclear flashes on the surface of the neutron star³, are sometimes observed^{4,6}, as well as quasiperiodic oscillations in the X-ray flux. The frequencies of the quasiperiodic oscillations depend on the spectral state of the source⁷⁻¹³, which manifests itself as three distinct spectral 'branches' in an X-ray colour-colour diagram. GX17+2 is also a variable radio source¹⁴⁻²⁰; there is no believable optical counterpart²¹⁻²³. We report here on simultaneous X-ray and radio observations which showed a connection between the spectral branches and the radio brightness. The 6-cm and 20-cm flux density increased by factors of 30 ± 5 and 40 ± 10 , respectively, as the X-ray state changed from the so-called 'flaring branch' to the 'horizontal branch'. We suspect that this X-ray/radio connection is common among bright low-mass X-ray binaries.

We observed GX17+2 in the X-ray and radio frequencies between 28 March and 5 April 1988 (Fig. 1). The X-ray observations were made with the Large Area Counter (LAC) of the satellite Ginga^{24,25}, with an area of $\sim 4,000 \text{ cm}^2$ (~ 1.5 to $\sim 37 \text{ keV}$). Almost all X-ray data were accumulated in twelve energy channels with a time resolution of 7.8 ms; the data were corrected for spacecraft attitude, background and deadtime. The

attitude is accurate to $\sim 3'$, which translates into a $\sim 5\%$ uncertainty in the source flux. Figure 1 shows light curves and the X-ray spectral hardness.

GX17+2 was observed in all three of the X-ray spectral branches seen so far, and the results are displayed as a colour-colour diagram in Fig. 2. The 'horizontal' branch (H) is actually vertical in Fig. 2, but the identification of this branch is fairly certain: it is evident from a hardness-intensity diagram of the data (not shown) and from the fact that the quasiperiodic oscillation (QPO) frequencies, ranging from $\sim 18 \text{ Hz}$ to $\sim 26 \text{ Hz}$ in this data, are correlated with the source intensity^{10,13}.

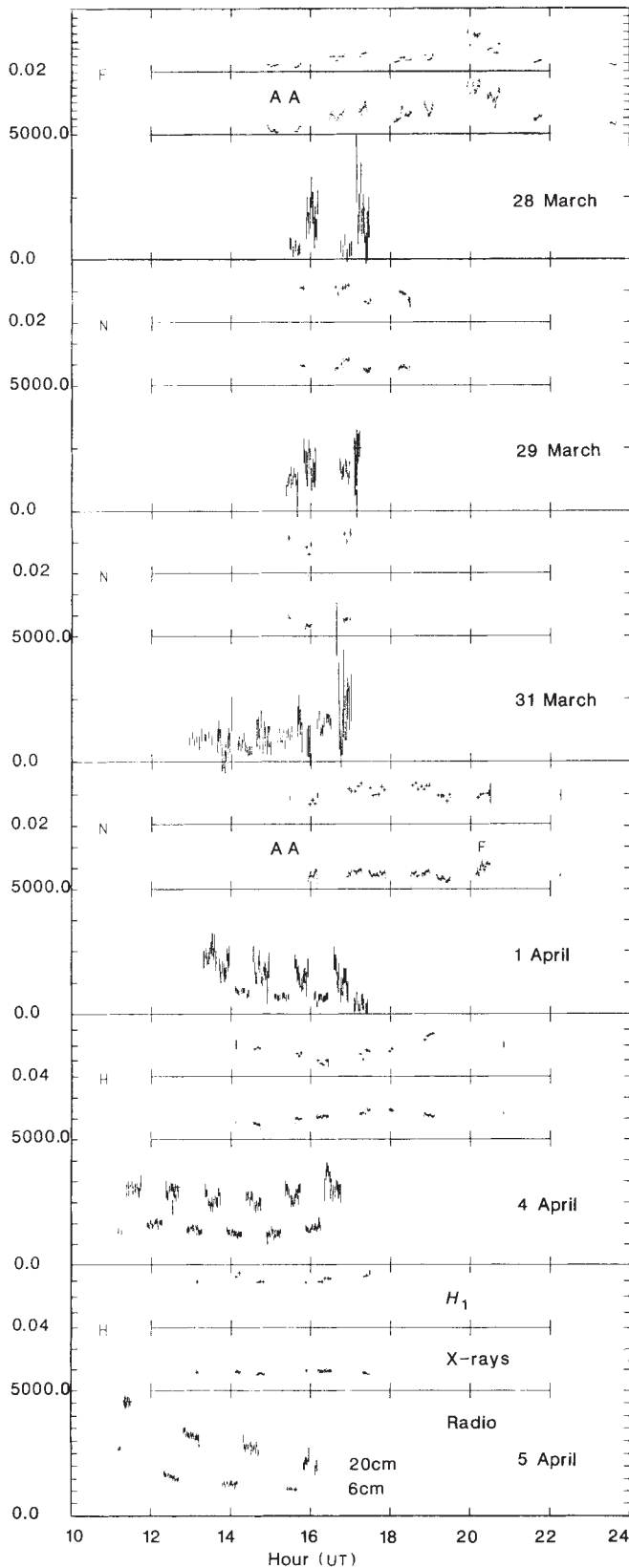
The radio observations (at 2, 6 and 20 cm) were carried out at the Very Large Array (VLA) which was in the C-configuration ($\sim 5''$ resolution at 6 cm). All 27 antennas were on-line most of the time. At each wavelength two adjacent bands of 50 MHz were observed. The flux densities were calibrated using the radio sources 3C286 and 3C48. Emission associated with GX17+2 was clearly detected at all three wavelengths. The 2-cm and 6-cm maps showed a weak background source $\sim 2'$ west of GX17+2. The 20-cm map contained a few sources and extended structure in the background. The difference between the flux densities of the two adjacent bands were taken as a measure for the error in the data. Variations in the atmosphere and the ionosphere are mostly corrected for in the calibration procedure, for time-scales $\geq 10 \text{ min}$. For details on the method of analysis, see, for example, ref. 26.

In Fig. 3 we plot the radio flux densities against the X-ray spectral hardness (H_1); there is a clear connection, in that the radio flux density increases as the X-ray spectral state changes from the flaring/normal branch to the horizontal branch.

The radio spectral index was ~ -0.62 when GX17+2 was bright and on the horizontal branch (see Fig. 1 legend). This, and the variability (up to $12 \pm 2\%$ in 10 min), indicate that the radiation is non-thermal. There is a possible analogy between the radio flaring in Cyg X-3 and GX17+2. The decay portion of a giant radio outburst of Cyg X-3 is well described as synchrotron radiation of an expanding cloud of relativistic electrons, as indicated by the rate of radio flux decay and the spectral index (-0.55) (refs 27-29). Synchrotron emission from a region with variable optical depth has also been observed in Cir X-1, LSI+61 303 and SS433 (refs 30-32). This mechanism may not, however, be the origin of the radio flares from the bright bulge source GX13+1 (ref. 26).

It is possible that the above connection between the radio flux and the X-ray spectral branches is common among low-mass X-ray binaries (LMXB). In addition to GX17+2, radio emission has been definitely detected from eight LMXBs: Sco X-1, Cyg X-2, Cyg X-3, GX5-1, GX13+1, A0620-00, Cen X-4, Cir X-1 and X2000+25 (refs 18-20, 28, 32, 33). Also, in addition to GX17+2, five other LMXBs have a known horizontal branch: Sco X-1, Cyg X-2, GX5-1, GX340+0 and GX3+1 (refs 34-37). The latter two sources have not been detected in the radio, but they probably spend little time, on average, on the horizontal branch^{35,36}. A0620-00, Cen X-4 (ref. 38) and X2000+25 in Vulpecula³⁹ are transient sources. To our knowledge, there are no published data on the spectral branch states of these sources. Thus, our suggestion that most LMXBs are radio bright on the horizontal branch and much less bright on the flaring branch, seems to be consistent with the available, albeit very limited, data base. (Hasinger and co-workers have performed simultaneous radio (VLA) and X-ray (Ginga) observations of Cyg X-2. They find a similar connection between the X-ray spectral branches and the radio flux (G. Hasinger, personal communication).)

Extensive radio observations were made of Sco X-1, some simultaneously with X-ray and/or optical observations⁴⁰⁻⁴³. Sco X-1 is radio quiet in its X-ray flaring branch, and it can be radio bright when it is X-ray quiescent (that is, in the normal or horizontal branches). This behaviour is consistent with our present results for GX17+2.



Although the power emitted in the radio is no more than $\sim 10^{-7}$ of that in the X-rays, its presence and correlation with the X-ray spectral branches could be important in understanding accretion in LMXBs. It may lead to an explanation of the branches and thereby perhaps also to the origins of the QPO (see, for example, refs 10 and 13). More simultaneous X-ray/radio observations are needed to evaluate our suggestion

◀ **Fig. 1** Plots of the X-ray hardness ratio, H_1 (see Fig. 2), the X-ray count rate (1.5–37 keV) and the radio flux densities (6 and 20 cm, which were observed alternately; our 2-cm data are not shown), all plotted as functions of time. Each panel represents one observing day. We indicated the plotting sequence in the bottom panel only. On the plots, one should distinguish the 6-cm from the 20-cm flux by the fact that the former is almost always lower and has a smaller error than the latter. The three labels (ordinate) in each panel indicate the values that correspond to the drawn abscissae: zero (mJy) for the radio data, 5,000 counts s^{-1} for the X-rays and 0.02 or 0.04 for H_1 . Consecutive tic marks represent changes of 2.5 mJy (radio), 1,000 counts s^{-1} (X-ray) and 0.005 (H_1). The X-ray spectral states are indicated: F, N and H stand for flaring, normal and horizontal branch, respectively; the letters A (28 March and 1 April) indicate the times that the source was near the F–N apex (see Fig. 2). Notice the change (to F) on 1 April. The flux density at 6 cm varied between 0.46 ± 0.08 mJy and 13.4 ± 0.1 mJy, and at 20 cm between 0.57 ± 0.14 and 22.8 ± 0.2 mJy. The 2σ upper limit on circular polarization (5 April, 20-cm data) is 5%. Spectral indices, α (defined as $S_\nu = \nu^\alpha$, where ν = frequency and S_ν = flux density), were determined for the times that 2-cm and 20-cm observations were made (the 6-cm flux was calculated by interpolation, using 20-min averages). The value for α between 6 and 20 cm was -0.42 ± 0.36 on 28 March, -0.06 ± 0.24 on 29 March, 0.03 ± 0.15 on 31 March and -0.63 ± 0.02 (and constant to within the error) on 1, 4 and 5 April. The spectral index between 2 and 6 cm was $< +0.22$ (2σ upper limit) on 28 March, 0.39 ± 0.15 on 29 March and -0.60 ± 0.04 on 5 April. The variance of α was used to determine the errors.

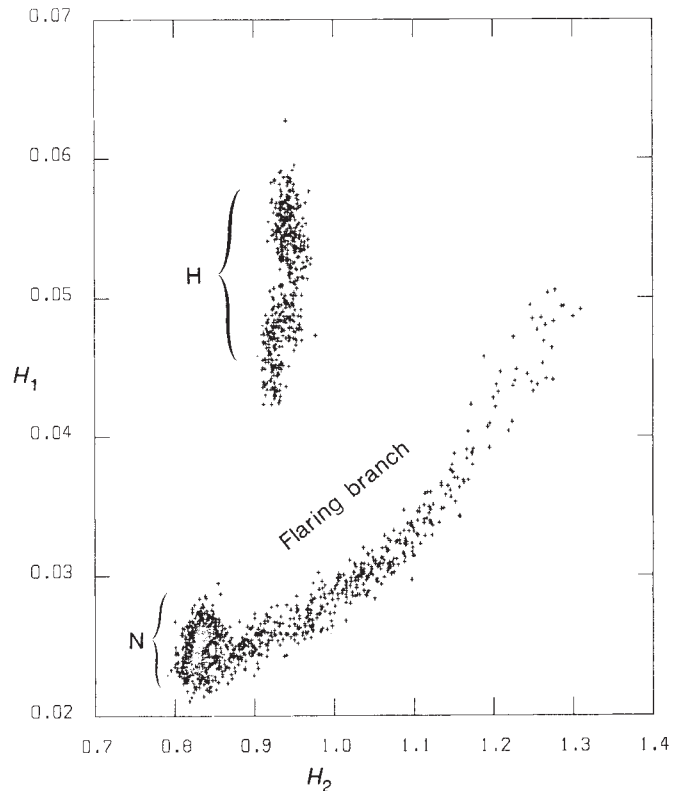


Fig. 2 Colour-colour diagram of the X-ray data. Each data point is a 32-s average. The ordinate and abscissa show the hardness ratios H_1 and H_2 , respectively, which represent the counting rate ratios in two energy bands: 14.0–18.6 keV/4.7–9.3 keV (H_1) and 4.7–9.3 keV/1.5–4.7 keV (H_2). We have indicated the X-ray spectral states: flaring branch, normal branch (N) and horizontal branch (H).

that the connection between the radio flux density and the X-ray spectral branches, observed for GX 17+2, also holds for many other LMXBs. If possible, observations should also be made simultaneously at other wavelengths.

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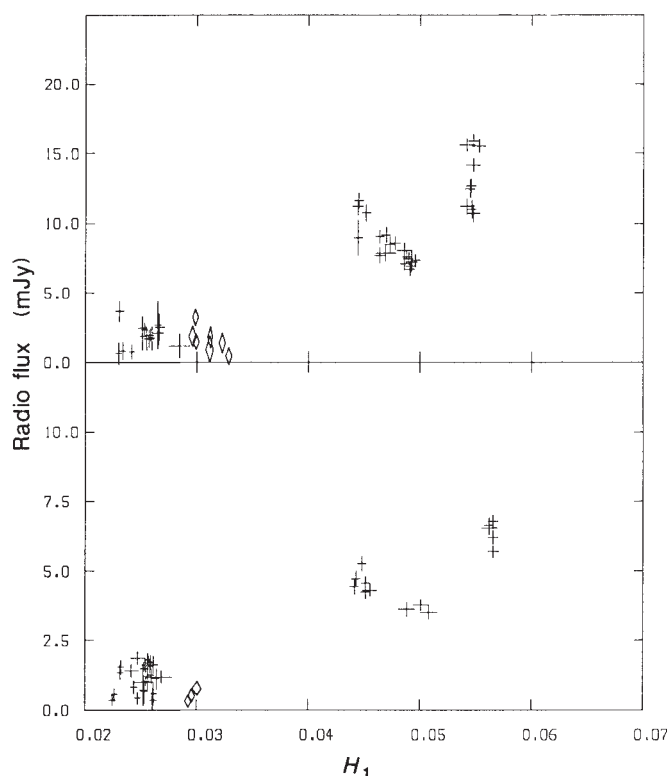


Fig. 3. Radio flux densities at 6 cm (bottom) and 20 cm (top) plotted against the X-ray hardness ratio, H_1 (see Fig. 2). The data points with values for H_1 in excess of 0.04 relate exclusively to the horizontal branch, whereas the data points with $H_1 < 0.04$ relate to the normal branch, the apex (indicated with an 'A' in Fig. 1) and the flaring branch. The flaring-branch data are indicated by diamonds. At 6 cm the flux densities appear somewhat higher on the normal branch than on the flaring branch, and they are by far the highest on the horizontal branch. The average 6-cm radio flux density (bottom panel) in the flaring branch was ~ 0.4 mJy; in the apex/normal branch it varied between ~ 0.6 and 1.6 mJy, and in the horizontal branch, between ~ 4 and 7.5 mJy. At 20 cm (upper panel) the flux density is also by far the highest on the horizontal branch.

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Sources of sulphur in forest canopy throughfall

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Atmospheric deposition of sulphate is the primary link between the atmosphere and acidification of soils and aquatic ecosystems¹. The flux of sulphate to forest soils can be measured in the water that drips from trees following the interception of rain to form stemflow and throughfall (the sum of which is designated here as TF). Enrichment of sulphur in TF over that found in rain is widely reported^{2–4}; sulphur sources include the wash-off of previously dry-deposited sulphate particles and SO₂ and the leaching of internal plant sulphur from foliage (termed foliar leaching)⁵. To quantify foliar leaching, we labelled mature trees in the field with radi sulphur and measured atmospheric sulphur concentrations and fluxes. Here we report that dry deposition provides $>85\%$ of the enrichment of sulphate in the TF flux to soils below three different tree species at low-elevation sites in the southeastern United States. This supports evidence from several forests that total atmospheric deposition of sulphate is strongly reflected in the measured flux in TF.

The enrichment of sulphate in TF is defined as net throughfall, or NTF (NTF equals flux in TF minus flux in rain). The foliar leaching portion of NTF, representing an internal cycling source of sulphur, should not be included in estimates of atmospheric inputs. Quantifying the sulphur flux in rain and TF is generally straightforward¹. In mountain and coastal areas, however, wind-driven rain and mist are not collected by rain samplers but impact on the forest and appear in TF. In these cases, NTF consists of both dry and interception deposition plus leaching. Many methods have been used to separate sulphate in NTF into leaching and deposition sources^{6–11}. Results have varied widely, with different sources apparently dominant in different studies⁵.

Stable isotopes have been used to quantify the sources of sulphur to plants in the field¹², and radioactive isotopes have been used to determine the fate of SO₂ deposited to plants in chambers^{3,13}. The contribution of foliar leaching to sulphate in TF below mature trees has now been quantified using a radioactive sulphur tracer. In June 1986 four deciduous trees (two red

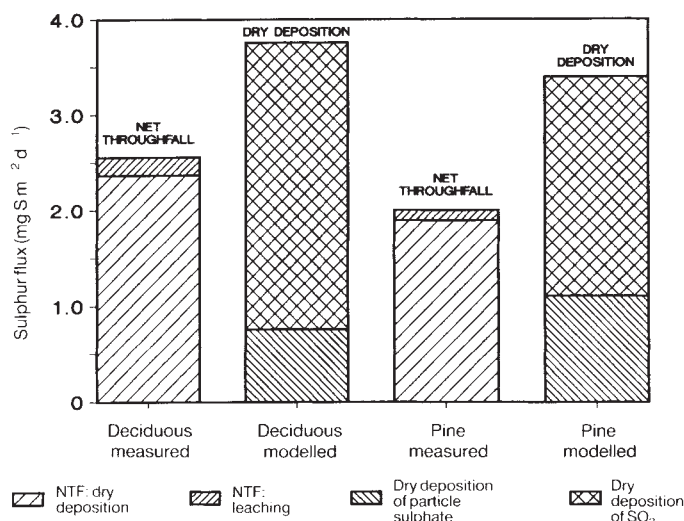


Fig. 1 Mean fluxes of sulphur in dry deposition to the canopies and in net throughfall (NTF) below the canopies of the eight study trees during the summer. NTF is defined as the flux of sulphate measured in throughfall plus stemflow minus that in incoming rain. NTF consists of foliar leaching (determined from sulphur radioisotope experiments) and dry deposition (determined by difference). The dry-deposition flux consists of model-derived estimates for particle sulphate and SO_2 .

maple (*Acer rubrum*) and two yellow poplar (*Liriodendron tulipifera*) were radiolabelled in the field with microcurie amounts of ^{35}S . Trees were labelled by building a collar around each trunk which was filled with distilled water. Chisel cuts were made 1 cm deep into the xylem, where the collar met the trunk. ^{35}S -labelled Na_2SO_4 was then added to the water which was drawn into the tree by xylem transport¹⁴.

In June 1987 the experiments were repeated on four loblolly pines (*Pinus taeda*). These trees were labelled by drilling a hole 10 cm deep into the trunk under a constant stream of 0.1 M KCl. Plastic tubing containing numerous holes on the bottom half was inserted into the hole, filled with 0.1 M KCl, joined to the bark with sealant, and connected to an elevated reservoir containing $\text{Na}_2^{35}\text{SO}_4$ in 0.1 M KCl. Trees absorbed the solutions in less than 12 hours with no loss of radioisotope through leaks. Use of 0.1 M KCl impeded resin flow which would have otherwise blocked entry of the isotope into the tree. All eight study trees were between 8 and 15 m tall. The sites are located in a rural forested area in eastern Tennessee^{4,15}, a region including several coal-fired power plants.

Between application of ^{35}S and the end of the growing season, we measured monthly concentrations of stable and radioactive sulphur in unwashed foliage¹⁶ and concentrations and fluxes in all rain (sampled in clearings) and TF (including stemflow) events⁸ occurring for 86 days (1986) and 100 days (1987). The fraction of ^{35}S and total S present as sulphate in leaves and needles was determined by extraction methods¹⁷ and was based on the total ^{35}S or stable S in the whole-leaf, respectively. Atmospheric concentrations of particle sulphate and SO_2 were measured from towers^{4,15,18,19}.

Both the deciduous and coniferous canopies had a strong effect on the concentration and flux of stable sulphate in TF relative to rain during the summer, resulting in an approximate doubling of the sulphate flux in rain (Table 1). At the pine forest, where summer and annual data are from the same year, the summer data are generally representative of the entire year with respect to the relative increase in sulphate below the canopies. The predominant form of airborne sulphur at these sites is SO_2 .

Foliar leaching contributed only a small percentage of the sulphur enrichment in TF below these canopies (Table 2). The fractional contribution of deposition and foliar leaching to TF sulphate was calculated from the mixing of two sources with different specific activities (that is, ^{35}S :total S)¹⁶. Whole-tree mass balances of ^{35}S confirmed minor leaching losses¹⁴. Hence, dry deposition wash-off accounted for 86 to 98% of the sulphate enrichment in TF beneath these eight trees.

Comparison of the fraction of ^{35}S and stable S as sulphate indicated that the added ^{35}S was a reasonable indicator of the existing forms of inorganic S in the leaf. The relative amount of ^{35}S as sulphate was consistently, but not significantly, higher than that for whole-leaf stable S in the deciduous trees¹⁶. In pine needles, the fraction of total foliar S as sulphate ($30\% \pm 4\%$) was similar to that for ^{35}S ($28\% \pm 3\%$ of the total ^{35}S was as sulphate). Greater leachability of foliar ^{35}S relative to total foliar S from the leaves would result in an overestimate of the specific activity in NTF, and, therefore, an underestimate of the relative contribution of dry-deposited sulphate to NTF.

Dry-deposited sulphur in industrialized areas occurs primarily as SO_2 and particle sulphate¹. Particle sulphate can be washed from foliage²⁰⁻²², but there are conflicting data on the mobility of dry-deposited SO_2 ^{3,13}. Assuming that the sulphate-enrichment in TF originates only from foliar leaching and wash-off of dry-deposited particles, the air concentrations of sulphate (Table 1) can be used to estimate a mean dry deposition velocity (V_d , dry deposition flux/air concentration) for particle sulphate to each canopy: $V_d \approx 0.8 \text{ cm s}^{-1}$ for the deciduous stand and $\approx 0.5 \text{ cm s}^{-1}$ for the pine. These V_d values are reduced if SO_2 also contributes to sulphate in TF.

Table 1 Concentrations and fluxes of stable sulphur for the summers of 1986 and 1987 in pine and deciduous stands

Time period	Forest site	Concentrations*				Fluxes†	
		Solutions		Air		Precipitation (mg S m^{-2})	Throughfall‡ (mg S m^{-2})
		Precipitation (mg S l^{-1})	Throughfall (mg S l^{-1})	Particles ($\mu\text{g S m}^{-3}$)	SO_2		
Summer 1986	Deciduous	0.95	1.8 (s.d. = 0.1)	3.6 (s.d. = 1.6)	6.2 (s.d. = 2.0)	260	480 (s.d. = 20)
1981-1982	Deciduous ⁴	0.87	2.4 (s.d. = 0.3)	2.7 (s.d. = 1.3)	7.5 (s.d. = 3.7)	1,100	2,600 (s.d. = 480)
Summer 1987	Pine	0.98	2.1 (s.d. = 0.1)	4.0 (s.d. = 2.0)	4.6 (s.d. = 2.0)	190	390 (s.d. = 20)
1986-1987	Pine ¹⁸	0.67	1.3 (s.d. = 0.2)	2.6 (s.d. = 1.6)	6.0 (s.d. = 3.5)	740	1,300 (s.d. = 200)

Recent annual data are included for comparison. Concentrations and fluxes (analysed as sulphate and SO_2) are reported as sulphur.

* Solution concentrations are volume-weighted means. Throughfall concentrations and fluxes include the standard deviations of the means (s.d.) from spatial replicates (4-6 for pine data and 4 for deciduous data). Air concentrations include standard deviations from all samples collected ($N = 14-28$ for summer, 25-49 for annual data).

† Hydrological fluxes in centimetres of rainfall are as follows: summer 1986 = 27.5, 1981-1982 annual = 128; summer 1987 = 19.7, 1986-1987 annual = 111. For the year, the water interception loss in the canopies of these forests is 7% for pine and 13% for deciduous.

‡ Throughfall fluxes (but not concentrations) include stemflow fluxes. Annual stemflow fluxes ranged from 0.3% (pine) to 14% (deciduous) of throughfall plus stemflow.

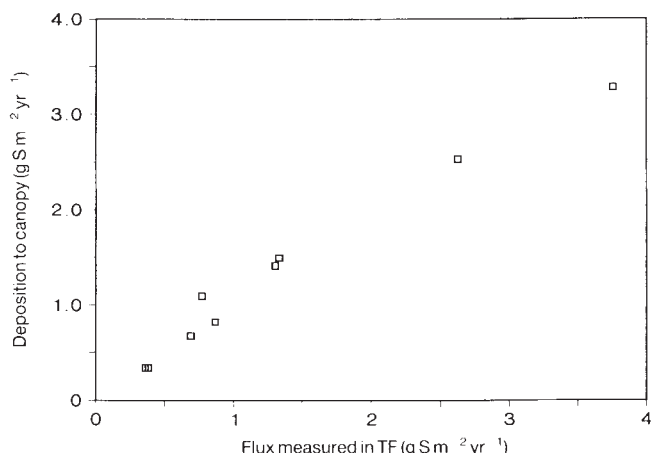


Fig. 2 The relationship between the estimated total atmospheric deposition of sulphate to forest canopies and the measured flux of sulphate in TF below the canopies at sites in the Integrated Forest Study^{15,19,27}. The forests include conifer and deciduous stands at elevations of 100 to 1,800 m in eastern and western North America. Wet deposition and dry deposition were determined as discussed in the text^{4,8,15,23}. All sites were sampled for at least 1 year (primarily 1986–1987, two from 1981–1984). The zero-intercept regression line has a slope of 0.94 (standard error = 0.04) and $r^2 = 0.96$.

Dry deposition of sulphate from particles and from oxidation of dry-deposited SO_2 was estimated for each site using weekly average air concentrations, hourly average meteorological data, and a resistance model²³ for V_d incorporating site-specific canopy structure data^{14–16,18}. Model predictions of total sulphate dry deposition exceed the flux of sulphate in NTF by factors of 1.6–1.8. Model predictions of particle dry deposition alone however, represent only 30–60% of the fluxes of sulphate measured in NTF (Fig. 1).

These comparisons indicate that the model overestimates dry deposition, that some dry-deposited sulphur is not washed from the canopy, or that both occur. Assuming that particle sulphate is readily washed from the canopy^{20–22} and that the model-derived V_d values are accurate, the data indicate that just over 50% of the dry-deposited SO_2 was washed from the deciduous canopies into TF during the course of our experiments and that just over 30% was washed from the pine (Fig. 1). Eddy correlation measurements of SO_2 dry deposition in the deciduous stand show that the model does overpredict deposition velocities for SO_2 by about 20% (ref. 18). If the actual flux of SO_2 is lower than that predicted here, the relative wash-off of SO_2 in TF may be higher than 30–50%. Field-cuvette experiments with ^{35}S -labelled SO_2 indicate that water can wash ~50% of dry-deposited SO_2 from loblolly pine needles during the summer¹³.

We reported that sulphate removed from the deciduous canopy in NTF consists of two fractions with different kinetics of removal, one indicating surface wash-off and the other diffusion from within the leaf^{4,8}. Results of the ^{35}S studies suggest that the surface source includes dry deposited particles and SO_2 deposited to the leaf cuticle, whereas the diffusing source includes internal plant sulphur and SO_2 dry-deposited through stomates to internal tissues. In this context, the term foliar leaching describes the loss of internal plant sulphur originating only from root uptake and translocation, which is a small fraction of NTF.

Longer-term data from these forests support minor foliar leaching. The annual mass balance of sulphate in the deciduous canopy (that is, the difference between the measured flux in TF shown in Table 1 and the measured wet deposition plus modelled dry deposition to the canopy⁴, where a positive difference represents foliar leaching) indicates that there is sufficient dry deposition of SO_2 and particle sulphate to account for most of

Table 2 Foliar ^{35}S concentrations, the fluxes of sulphate (as S) in net throughfall (NTF)*, the specific activity (SA)[†] of ^{35}S in foliage and NTF, and the contribution of foliar leaching to the sulphate flux in NTF beneath the study trees

	Foliar ^{35}S ($\mu\text{Ci g}^{-1}$)	NTF sulphate flux (mg S m^{-2})	SA ($\mu\text{Ci } ^{35}\text{S mg}^{-1} \text{S}$)		Contribution of foliar leaching [‡] (%)
			Foliar	NTF	
Poplar 1	0.0041	240	0.0014	1.5×10^{-4}	11
Poplar 2	0.0098	200	0.0056	1.7×10^{-4}	3
Maple 1	0.0058	230	0.0051	7.9×10^{-5}	1.5
Maple 2	0.0003	200	0.0004	5.7×10^{-5}	14
Mean for deciduous trees					7.4 (s.d. = 6.1)§
Pine 1	0.0045	180	0.0042	2.2×10^{-4}	5.2
Pine 2	0.0023	180	0.0035	1.2×10^{-4}	3.4
Pine 3	0.0049	220	0.0049	3.8×10^{-4}	7.8
Pine 4	0.0043	210	0.0056	2.5×10^{-4}	4.5
Mean for pines					5.2 (s.d. = 1.9)§

* NTF is the enrichment of sulphate in throughfall over rain, computed as flux in throughfall (including stemflow) minus flux in precipitation.

† SA, ratio of ^{35}S to total S.

‡ Foliar leaching = (SA TF)/(SA leaf) $\times$ 100.

§ s.d., standard deviation of the mean.

the sulphate enrichment in TF. Hence, foliar leaching accounts for ~5% of the total annual flux of sulphate in TF below this canopy. These conclusions are supported by previous work on ecosystem-level sulphur cycling²⁴ and tree fertilization studies²⁵ at this forest. The annual mass balance of sulphate in the pine canopy suggests that about 70% of the annual dry-deposited SO_2 is washed from the canopy in TF and that annual foliar leaching is insignificant relative to total TF below this pine canopy¹⁹.

These data indicate that SO_2 wash-off is more complete during winter than summer, leading to higher annual wash-off rates of SO_2 than summer data alone would indicate. This difference in seasonal wash-off may be attributed to the absence of foliage from the deciduous canopy and to the longer stomatal closure time in pines during the winter. Both result in proportionally more dry deposition of SO_2 to external plant surfaces and less to internal leaf membranes. The mass balance data also indicate that the relative contribution of foliar leaching of ^{35}S in TF during summer appears representative of the annual contribution of foliar leaching of stable sulphate to TF for both canopies.

Our results indicate that foliar leaching contributes a small percentage of the enrichment of sulphate in TF below three different forest tree species in this region. The dominant source of sulphate in NTF at these sites is wash-off of dry-deposited particle sulphate and of SO_2 that is oxidized to sulphate within the forest canopy following deposition. This finding contradicts results of a study of a Scots pine (*Pinus sylvestris*) stand in Great Britain (with similar total atmospheric S deposition) that identified foliar leaching as the primary cause of enrichment of sulphate in TF (ref. 11).

We expect to find that the relative importance of leaching and dry deposition to the enrichment of sulphate in forest TF is influenced by the local environment. For example, the contribution of foliar leaching to TF should increase in relative importance at background sites with low sulphur dry deposition, particularly if foliar leaching is uniformly low in absolute terms for many forests²⁴. Although our results may not extend to background areas, the annual sulphate deposition rate in the region of our studies ($1\text{--}3 \text{ g S m}^{-2}$) (refs. 4 and 18) is representative of values for much of Europe²⁶ and North America¹.

For forests in these regions, low foliar leaching has important implications for deposition measurement strategies. A recent study of atmospheric deposition to nine forests in North America^{15,19,27} illustrates the value of TF measurements. The annual atmospheric deposition rates of sulphur to these forests are very similar in magnitude to the measured annual fluxes of

sulphur in TF (Fig. 2). Considering the uncertainty in such deposition estimates, we conclude that measurements of TF fluxes of sulphate below forest canopies can provide a useful means of assessing trends in atmospheric sulphur inputs to forested ecosystems in industrialized nations, where sulphur deposition is an important concern.

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Role of complexation processes in cadmium mobilization during estuarine mixing

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Complexation reactions with ligands in solutions¹ and on solid surfaces^{2–4} are essential features of the biogeochemical cycling of trace metals such as cadmium⁵. Field studies^{6–8} and laboratory experiments^{8–11} show that cadmium can be mobilized from riverine particulate matter when river water mixes with sea water. Although this mobilization is often attributed to the formation of cadmium chloro complexes in solution^{6,9,11}, the role of these competitive complexation processes has not been quantified. Moreover, there is controversy concerning the reversibility of the interaction of

cadmium with particulates^{11–13}, which is an essential factor controlling the amount of cadmium that can be mobilized. Here we report the results of laboratory experiments and equilibrium calculations which demonstrate that cadmium partition between suspended particles and solution is completely reversible, and that complexation reactions in solution can completely account for the amount of cadmium released from suspended particles. Apparently, this toxic metal is not irreversibly fixed on particles, but can be released in response to changes in the aquatic environment. This may have important implications for the bioavailability of particle-bound cadmium.

Our experiments were designed to investigate both the reversibility of cadmium sorption on riverine suspended particles in fresh water, and the desorption process of pre-adsorbed cadmium in more saline water. Conditions after equilibration were close to those in fresh-water environments in industrialized regions. Cadmium was adsorbed on suspended particles from the River Rhine (sampled at Lobith, The Netherlands) at low initial concentrations in fresh water. Following adsorption equilibration, the particles were separated and re-suspended both in the same fresh water solution without dissolved cadmium (as this provides the most direct test of adsorption reversibility)¹³ and in mixtures of fresh water and sea water, and cadmium desorption in these solutions was studied.

The kinetics of adsorption and desorption are shown in Fig. 1. In adsorption, an initial period of rapid uptake of cadmium from solution is followed by slow uptake processes which continue over several days. Cadmium was released rapidly from the solids during the early stages of desorption, and continued slowly over the entire reaction period. The trends in Fig. 1 indicate slow sorption processes with characteristic times of days to weeks, such as those observed recently for trace-metal sorption on natural particles^{12–14}. These slow kinetic processes are important, because such timescales over which sorption processes may be reversible¹³ are similar to the residence times of particles and water in many estuaries^{5,6,15}.

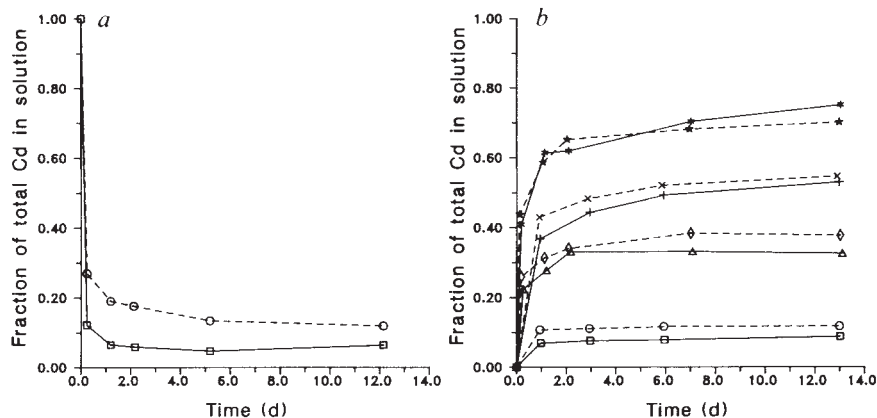
Figure 2 shows isotherms of cadmium adsorption and desorption in fresh water after an equilibration period of 12 days. There is no significant hysteresis between the two isotherms; the distribution of cadmium between the particles and the solution, after adsorption, is restored after desorption under similar conditions. This demonstrates that the sorption process of cadmium on these natural suspended particles is completely reversible.

It is important to know whether trace metals interact reversibly with suspended particles because the extent of the reversibility controls the amount of metal that is released to the solution during desorption. Irreversibility has, however, been reported for nickel and cobalt sorption on the clay mineral montmorillonite¹⁶ and for cadmium on natural sediments¹¹. More recently cadmium has been shown to interact reversibly with illite in fresh water¹³ and with natural suspended particles in sea water¹², which is in accordance with our present findings. The conflicting behaviour may in some cases be due to the recognition only recently that these systems exhibit slow sorption processes (compare Fig. 1) which require relatively long desorption times for reversibility to be apparent¹³.

When particles with pre-adsorbed cadmium are suspended in fresh water/sea water mixtures, the slopes of the desorption isotherms decrease strongly with increasing salinity (Fig. 3a). Clearly, as salinity increases, the distribution of cadmium between the particles and the solution changes dramatically and the metal is released progressively from the solids. This salinity-induced desorption behaviour of cadmium agrees with earlier findings, both in the laboratory^{8–11} and in the field^{6–8}. In most of these studies this mobilization was attributed to the formation of Cd-chloro complexes in solution, which are highly stable. The main question to be addressed, however, is whether the changing speciation of cadmium in the estuarine mixing zone can quantitatively explain the redistribution of the trace metal

Fig. 1 The fraction of cadmium in solution as a function of time. *a*, Adsorption on suspended particles from the River Rhine in fresh water. *b*, Desorption of pre-adsorbed cadmium from these particles at different salinities. Solid and dashed lines represent samples with an initial concentration of 1 and 20 $\mu\text{g Cd(II)} \text{ l}^{-1}$ respectively. Salinities in *b* are as follows: $\circ$, $\square$, 0.0‰; $\diamond$, $\triangle$, 2.0‰; $\times$ and $+$, 5.9‰; $\star$ and $*$, 35.5‰.

Methods. Natural suspended solids were collected from the Rhine river near Lobith (The Netherlands) in October 1987 using a continuous flow centrifuge. For the chemical and mineralogical composition of this material see ref. 24. Mineralogy and organic matter content are approximately: 8% calcite, 2% dolomite, 2.5% iron hydroxide, 24% quartz, 3% kaolinite, 20% illite, 10% smectite, 4% microcline, 3% albite and 18% organic matter²⁴. After storage at 4 °C in the dark for about 3 weeks, wet solids were suspended in a simple fresh-water solution containing only Ca^{2+} as competing cation, HCO_3^- as a natural pH buffer and NO_3^- (non-complexing) at an ionic strength of $3 \times 10^{-3} \text{ M}$ and a pH of 7.85 (ref. 13). To pre-equilibrate the solids with the electrolyte solution and to remove exchangeable metals, suspensions were transferred to dialysis tubing and were dialysed for 2 weeks in regularly refreshed electrolyte solution. Sorption experiments were performed in acid-cleaned 110-ml Teflon (PTFE) and 90-ml Teflon (PFA) reaction vessels. Teflon materials were used to minimize sorption of cadmium on vessel walls at the low concentrations used ($<3\%$ at the $1\text{-}\mu\text{g l}^{-1} \text{ Cd(II)}$ level over the 12-day reaction period). The electrolyte solution, solids and standard solution of cadmium, labelled with 8.5-kBq ^{109}Cd ($37 \text{ MBq } \mu\text{g}^{-1} \text{ Cd}$), were added as described in ref. 13. Samples had an initial volume of 80 ml with a particle concentration of 100 mg l^{-1} and initial Cd(II) concentrations ranging from 1 to $20 \mu\text{g l}^{-1}$. Four batches of duplicate samples for each of the five initial Cd(II) concentrations were equilibrated for 12 days under continuous shaking so that the adsorption process could be followed. At intervals during equilibration, two 2-ml subsamples were taken for monitoring the pH and for γ -counting the ^{109}Cd activity of the liquid phase in 1 ml of the clear supernatant after 30-min centrifugation at $2 \times 10^4 \text{ m s}^{-2}$. The aqueous and sorbed cadmium concentrations were calculated as described in ref. 13. After adsorption equilibration and centrifugation at $2 \times 10^4 \text{ m s}^{-2}$, the cadmium-containing solids were separated from solution. The particles were re-suspended in equal volumes of (1) the same electrolyte solution without Cd(II) , (2) a 17:1 mixture of the electrolyte solution and filtered Atlantic surface water (2.0‰ salinity), (3) a 5:1 mixture of the same solutions (5.9‰ salinity), and (4) pure Atlantic surface water (35.5‰ salinity). The desorption process was studied in the same reaction vessels during equilibration for 12 days under continuous shaking. Subsampling and pH monitoring were as described above.



between the solid phase and the solution. Otherwise, additional processes must be involved, such as competition between major seawater cations (Ca^{2+} , Mg^{2+}) and the trace-metal cation for sorption sites on the suspended particles, or changes in the surface charge of the particles, as have been suggested to cause Pb(II) mobilization during estuarine mixing¹⁵.

The speciation of cadmium in the various fresh water/sea water mixtures that we used was calculated with a chemical equilibrium model¹⁷ based on the WATEQF program¹⁸. Stability

constants of the cadmium species used in the model are listed in Table 1. The results of the equilibrium calculations are summarized in Table 1. The Cd-chloro complexes determine most of the cadmium speciation at increased salinity. In Fig. 3b we have re-plotted the sorption data at the different salinities (Figs 2 and 3a) as a function of the free Cd^{2+} activity in solution. This isotherm plot displays a single linear relationship (correlation coefficient = 0.984). We conclude, therefore, that the desorption behaviour of cadmium is regulated entirely by the decrease-

Table 1 Stability constants of reactions used in the cadmium speciation model and results of the speciation calculations at different salinities

Reaction	log (K)	Ref.	A†	Calculated speciation % of total dissolved Cd(II)*			
				B†	C†	D†	
$\text{Cd}^{2+} = \text{Cd}^{2+}$			76.8	17.7	6.9	0.9	
$\text{Cd}^{2+} + \text{OH}^- = \text{Cd(OH)}^+$	3.92	1	0.3	0.1	0.4	—	
$\text{Cd}^{2+} + 2\text{OH}^- = \text{Cd(OH)}_2$	7.65	1					
$\text{Cd}^{2+} + 3\text{OH}^- = \text{Cd(OH)}_3^-$	8.70	1					
$\text{Cd}^{2+} + 4\text{OH}^- = \text{Cd(OH)}_4^{2-}$	8.65	1					
$\text{Cd}^{2+} + \text{F}^- = \text{CdF}^+$	1.0	20					
$\text{Cd}^{2+} + 2\text{F}^- = \text{CdF}_2$	1.4	20					
$\text{Cd}^{2+} + \text{Cl}^- = \text{CdCl}^+$	2.0	20	—	45.7	48.5	31.7	
$\text{Cd}^{2+} + 2\text{Cl}^- = \text{CdCl}_2$	2.6	20	—	4.7	13.7	44.7	
$\text{Cd}^{2+} + 3\text{Cl}^- = \text{CdCl}_3^-$	2.4	20	—	—	0.6	10.0	
$\text{Cd}^{2+} + 4\text{Cl}^- = \text{CdCl}_4^{2-}$	1.7	20	—	—	—	0.7	
$\text{Cd}^{2+} + \text{SO}_4^{2-} = \text{CdSO}_4$	2.45	1	—	3.1	2.3	0.6	
$\text{Cd}^{2+} + 2\text{SO}_4^{2-} = \text{Cd(SO}_4)_2^{2-}$	3.44	1					
$\text{Cd}^{2+} + 3\text{SO}_4^{2-} = \text{Cd(SO}_4)_3^{4-}$	3.09	1					
$\text{Cd}^{2+} + 4\text{SO}_4^{2-} = \text{Cd(SO}_4)_4^{6-}$	-0.72	1					
$\text{Cd}^{2+} + \text{CO}_3^{2-} = \text{CdCO}_3$	4.0	3	0.8	0.3	1.1	—	
$\text{Cd}^{2+} + \text{CO}_3^{2-} = \text{CdCO}_3(\text{s})$	11.3	3					
$\text{Cd}^{2+} + 2\text{OH}^- = \text{Cd(OH)}_2(\text{s})$	14.3	20					

Activity coefficients for all aqueous species were calculated with the Davies equation²¹. Stability constants for aqueous species of the major elements were taken from ref. 18 and updated with data from refs 22 and 23.

* % species = activity species/total dissolved Cd(II) concentration.

† A = 0‰ salinity, pH 7.69; B = 2.0‰ salinity, pH 7.88; C = 5.9‰ salinity, pH 7.86; D = 35.5‰ salinity, pH 8.24.

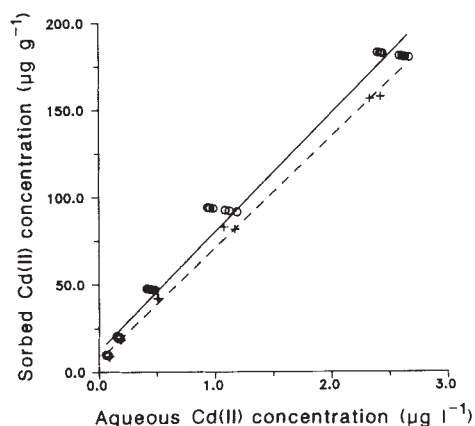


Fig. 2 Sorption isotherms describing the distribution of cadmium between particles and solution in fresh water after adsorption on, and desorption from, suspended particles from the Rhine (12 days equilibration). $\circ$ —adsorption, $pH = 7.85 \pm 0.02$; $+$ —desorption, $pH = 7.69 \pm 0.04$. The overlap of the two sets of data implies that the sorption process is completely reversible. Solid and dashed lines are linear fits to adsorption and desorption respectively.

ing free Cd^{2+} activity in solution, through the combined effect of increasing cadmium complexation (mainly by Cl^-) and increasing ionic strength during the mixing of fresh water and sea water. This strongly suggests that the free Cd^{2+} activity determines the partition of cadmium between the particles and the solution. When, during estuarine mixing, the Cd^{2+} activity is reduced by chloride complexation and ionic strength, the reversibility of the interaction of cadmium with these particles causes the metal to be redistributed in a manner that is described by a single sorption isotherm. The system can be viewed as direct competition for Cd^{2+} ions between ligands at particle surfaces (such as hydroxo- or organic functional groups) and ligands (mainly Cl^-) in solution. These conclusions are in general agreement with the results of laboratory mixing experiments using filtered sea water and unfiltered river water^{8,10}. In these experiments, most of the observed mobilization of particle-bound cadmium occurs at salinities up to ~6–12‰; that is, in the region where the Cd^{2+} activity decreases most sharply (compare Table 1).

Trace-metal uptake by biota (which is relevant to toxicity) is also known to be related to the activity of the free aquo metal

ion and may, to a certain extent, be treated as a series of complexation reactions (ref. 19 and references therein). Thus biota are also involved in the competitive complexation reactions that affect the behaviour of cadmium in the environment. The fact that cadmium interacts reversibly with particles and can be mobilized in proportion to its decreasing free aquo ion activity implies that the particle-bound fraction of this toxic metal may ultimately become available to aquatic organisms.

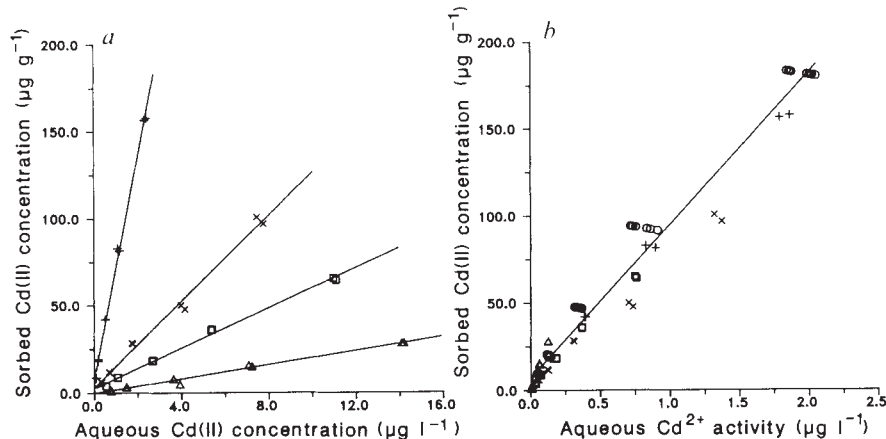
Although increased cadmium mobilization is generally observed during the mixing of fresh water and sea water (ref. 6 and references therein), in some cases both dissolved and particulate cadmium concentrations are observed to decrease⁵. The Rhine estuary is a typical example of the latter situation, which has been attributed to mixing of riverine particles with less contaminated marine-derived particles in a turbidity maximum⁵. Our laboratory data show that during this process cadmium must first desorb from the pre-existing riverine particles, in reversible response to the efficient competition for Cd^{2+} by dissolved chloride, and must then be re-adsorbed on the newly introduced marine particles. Apparently, cadmium is not irreversibly fixed on particles but has the potential to respond reversibly to changes in the (estuarine) environment.

We have shown that, under controlled laboratory conditions, cadmium reacts reversibly with surfaces of natural riverine particles and desorbs in proportion to its complexation in solution. Although our laboratory conditions were kept close to those in the natural environment, the aqueous $Cd(II)$ concentrations that we used range from values which are close to those found in most rivers and oceanic coastal waters up to values which are 1–2 orders of magnitude higher.

In individual estuarine systems, (lateral) variations in factors such as pH , redox conditions and the nature and amount of organic material may have additional (positive or negative) effects on the mobilization of cadmium. Recognition of the quantitative role that complexation processes in solution can play in the mobilization of cadmium during estuarine mixing may further our understanding of the complex behaviour of cadmium in natural estuarine environments. Our findings suggest that the bioavailability of particle-bound cadmium may be greater than is generally believed⁵.

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Fig. 3 Sorption isotherms describing the distribution of cadmium between particles and solution after desorption of pre-adsorbed cadmium at different salinities (12 days equilibration). *a*, Desorption data plotted as a function of the $Cd(II)$ concentration in solution. *b*, Adsorption and desorption data re-plotted as a function of the Cd^{2+} activity in solution (calculated with a chemical equilibrium model). $+$ —salinity 0‰, $pH = 7.69 \pm 0.04$; $\times$ —salinity 2.0‰, $pH = 7.88 \pm 0.04$; $\square$ —salinity 5.9‰, $pH = 7.86 \pm 0.02$; $\triangle$ —salinity 35.5‰, $pH = 8.24 \pm 0.02$; $\circ$ —adsorption data, salinity 0.0‰, $pH = 7.85 \pm 0.02$. It appears that during the mixing of fresh water and sea water, the progressive complexation of cadmium (which decreases the Cd^{2+} activity) completely accounts for the amount of cadmium that is released from the solids. To investigate the possibility of particle coagulation at increasing salinity, we measured the grain size distribution of the suspended particles in a parallel experiment with a Malvern 3600 D Particle Sizer. We observed no significant changes in the grain size distribution over 15 size fractions between 1.2 and 118 μm . The median grain size of the particles in all suspensions was $18.2 \pm 1.8 \mu m$.



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Geotherms in the Pacific Ocean from laboratory and seismic attenuation studies

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Seismic methods have been used extensively to measure attenuation coefficients in various regions of the Earth. Attenuation is inversely related to the quality factor Q , which may be measured directly. A low-velocity and high-attenuation zone in the upper mantle is commonly reported^{1–3}. Until now, there has been no direct means to derive thermal structures from such seismic investigations. Recently, Q values have been determined in the laboratory for a dry peridotite, an upper mantle rock, for pressures and temperatures of up to 0.73 GPa and 1,280 °C respectively⁴. The results indicate a quantifiable relation between Q and temperature. Here we use these results to derive geotherms from Q measurements for a region beneath the Pacific Ocean. The geotherms are consistent with temperatures obtained from heat flow observations^{5,6}, and imply that the oceanic upper mantle is rather dry. In asthenosphere older than 40 Myr, geotherms for this region are sub-solidus and no partial melt is expected.

A seismic attenuation coefficient, or quality factor, for compressional waves, Q_p , has been determined in the laboratory for a dry peridotite (spinel lherzolite from British Columbia) at high pressures (0.2–0.73 GPa) and high temperatures (950–1280 °C)⁴, using pulse transmission and spectral ratio techniques^{4,7}. Pressures were generated by an internally heated gas-media (pure argon) high-pressure apparatus. Seismic coupling between the sample (1.6 cm long) and transducer was made by a buffer rod of mullite. The sample and the buffer rods were tightly packed into a platinum tube, which was placed inside a

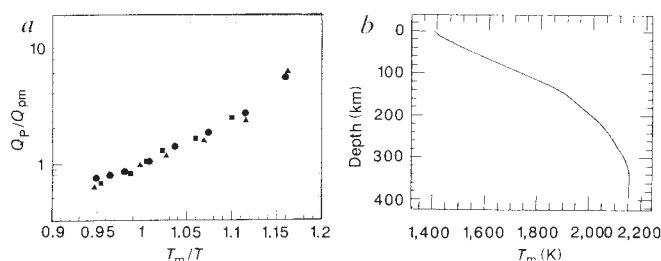


Fig. 1 *a*, Homologous temperature dependence of Q_p determined at high temperatures (950–1,280 °C) and high pressures. Symbols denote different pressures: ●, 0.73 GPa; ■, 0.48 GPa; ▲, 0.20 GPa. T and T_m are given in kelvins. *b*, Dry peridotite solidus as a function of depth (original data from ref. 9).

platinum wire furnace. After the measurements, microscopic observations revealed no oxidation of the sample. The measurement of attenuation covered the frequency range 60–880 kHz. Two independent measurements were carried out, one with the entire sample assembly and the other with only the buffer rod. Identical procedures were used for both assemblies, so as to distinguish attenuation in the buffer rod. Thus, the travel-time delay and small attenuation effect in the buffer rod were calibrated and accurate Q_p values in the sample could be obtained. Detailed descriptions of the method of measurement are reported elsewhere^{4,7}.

These laboratory results show that Q_p is highly temperature-dependent even below the solidus, and, more importantly, that a linear relation exists between $\log(Q_p)$ and the solidus temperature, T_m , of the peridotite. If Q_p is normalized by the value at the solidus temperature, Q_{pm} , and temperature is normalized by the solidus temperature, the data at three different pressures have a single trend (Fig. 1*a*). Q_p/Q_{pm} is pressure-dependent only through the pressure dependence of T_m , that is, the homologous temperature dependence. This observation allows us to extrapolate laboratory results to higher pressures (greater depths), requiring only a knowledge of the solidus as a function of pressure. Previous attenuation measurements⁸ made at 1 atm pressure could not be used at depth in the Earth, because the effect of pressure on Q was not known. Without high pressure, which closes cracks, systematic high-temperature measurements cannot be reliably made for polycrystalline rocks.

The relationship observed in Fig. 1*a* can be expressed as

$$Q_p/Q_{pm} = \exp[g(T_m/T - a)] \quad (1)$$

where g and a have the respective values 6.75 and 1.00 for $T_m/T < 1$, 8.47 and 1.00 for $1 < T_m/T < 1.08$ and 13.3 and 1.03 for $T_m/T > 1.08$ (ref. 4). Q_{pm} is a linear function of pressure or of pressure divided by the solidus temperature:

$$Q_{pm} = Q_{01} + p/p_0 \quad (2)$$

$$Q_{pm} = Q_{02} + pV_0/RT_m \quad (3)$$

where p is the pressure and R is the gas constant. Q_{01} , Q_{02} , p_0 and V_0 have the values 3.5, 3.4, 0.073 GPa and $170 \text{ cm}^3 \text{ mol}^{-1}$, respectively⁴. To extrapolate Q_p to a mantle depth, we employ the solidus of a dry peridotite determined up to 14 GPa (≈ 400 km depth) by Takahashi⁹ (Fig. 1*b*). (The composition of peridotite in this study is very similar to that used by Takahashi; minor compositional variations do not cause a significant difference (less than $\sim \pm 20$ °C) in the solidus⁴.) Because T_m gradually increases with increasing pressure (Fig. 1*b*), equations (2) and (3) are very similar in terms of the pressure dependence of Q_{pm} , deviating slightly only at higher pressure ($Q_{pm} = 140$ and 99 from equations (2) and (3), respectively, at 10 GPa). But even at high pressure, temperature estimates derived from two equations do not show significant disparity (see later; compare Fig. 3). Although no experimental Q_p data are available at higher pressures, homologous temperature relations similar to equation

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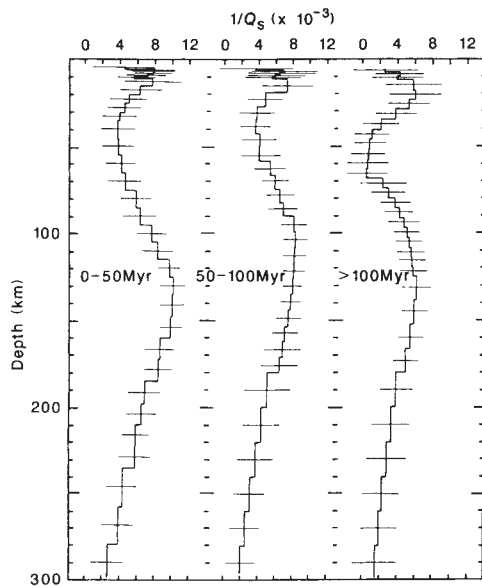


Fig. 2 Seismic Q_s structure for three different ages beneath the Pacific Ocean (after Canas and Mitchell²).

(1) have been established empirically for diffusion coefficients in metals^{10,11}, for creep rate of rocks^{12,13} and for seismic velocity and partial melt fraction in peridotites¹⁴. Changes in chemical composition and pressure that change T_m do not seriously change g values for diffusion coefficients. We may therefore extrapolate equation (1) to greater depth in the mantle. The dependence of Q_p on Q_{pm} is much weaker than its dependence on T_m/T (see equation (1)), so any inaccuracy from extrapolation of equation (2) or (3) causes less significant errors in temperature estimates than do inaccuracies resulting from errors in T_m . It has been shown that the extrapolation of equations (1) and (2) (or (3)) to upper-mantle conditions gives Q_p values comparable to seismically determined Q values for this region⁴.

A geotherm can be determined from seismic Q structure by rearranging equation (1):

$$T = T_m / [a + \ln(Q_p/Q_{pm})/g] \quad (4)$$

In this study, we have used $g = 13.3$ and $a = 1.03$ for temperature estimates beneath the Pacific Ocean, because the resulting temperature corresponds to $T_m/T > 1.08$ (see later; compare Fig. 3). For the quality factor of shear waves, Q_s , determined from seismic study, we employ the assumption $Q_p/Q_s = 2.25$ (ref. 15). We calculate pressure as a function of depth using the pressure-depth conversion reported by Gilbert and Dziewonski¹⁶, and thus we obtain T_m (Fig. 1b) and Q_{pm} as functions of depth. Therefore, given the Q -depth profile from seismic studies, equation (4) determines a geotherm. Although the laboratory measurement used an ultrasonic technique (frequencies above 60 kHz), the grain size was chosen to make Q values determined at laboratory frequencies comparable with those in the Earth at seismic frequencies⁴. In the laboratory, the grain size was in the range of 3–800 μm , which is much smaller than grain sizes observed in rocks from the upper mantle (~ 3 mm (ref. 17)). The laboratory results⁴ show that grain boundary relaxation¹⁸ is a possible attenuation mechanism in the peridotite sample. Smaller grain size causes higher relaxation frequencies of Q (refs 18, 19). Thus, higher frequencies should be used in the laboratory than in seismic studies to obtain comparable Q values. Leak¹⁹ shows that relaxation frequency is inversely proportional to the square of the grain size for polycrystalline iron, implying a factor of up to 10^6 ($= (3 \text{ mm}/3 \mu\text{m})^2$) difference between the relaxation frequencies of the laboratory and the mantle. In this case, frequencies of up to 1 MHz should be used in the laboratory to determine Q values that are comparable to

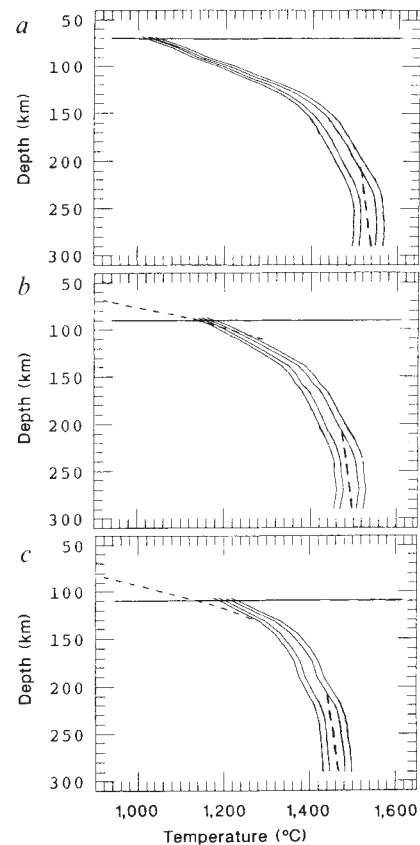


Fig. 3 Geotherms derived from laboratory and seismic Q measurements (solid curves) and from heat flow (light dashed line), and an adiabatic temperature gradient (heavy dashed line). The thin horizontal line denotes the bottom of the lithosphere. Geotherms derived from two Q_{pm} values (equations (2) and (3)) are shown, and are presented in terms of maximum and minimum estimates of temperature, allowing for a $\pm 15\%$ error in the laboratory Q . a, 0–50 Myr; b, 50–100 Myr; c, >100 Myr.

seismic Q values obtained at 1 Hz. In the laboratory measurements⁴, no frequency dependence of Q was resolved in the range of 60–880 kHz. A wave attenuation theory proposed by Kjartansson²⁰ suggests that Q is independent of frequency; this theory provided a good description of the attenuation measured in rocks. Any frequency dependence of Q would be manifest as a discrepancy between the temperature at the base of the oceanic lithosphere derived from Q and that calculated from heat flow. No such discrepancy is observed; the geotherm derived from the surface heat flow is consistent with the temperature derived from Q (Fig. 3).

We compare the laboratory results with seismically determined Q structures to determine the geotherm in the upper mantle. Seismic surface wave studies can be used to determine Q structures in regions of the upper mantle. Q structure as a function of plate age has been determined by Canas and Mitchell² for the Pacific Ocean (Fig. 2) and by Chan *et al.*³ for the Iceland Plateau. Applying equation (4) to the data in Fig. 2, we obtain the geotherms shown in Fig. 3. Because the laboratory results determined at high homologous temperature ($0.94 < T_m/T < 1.16$) correspond to conditions in the asthenosphere, we restrict the geotherms to beneath the bottom of the lithosphere. We estimate lithospheric thickness from seismic studies^{21,22}. The average ages of the three regions in Fig. 3 are estimated to be about 40, 75 and 125 Myr (ref. 2). Even the maximum estimate of temperature in these Pacific regions is well below the solidus of dry peridotite (Fig. 1b), so no partial melt is required at any depth in the three regions under volatile-

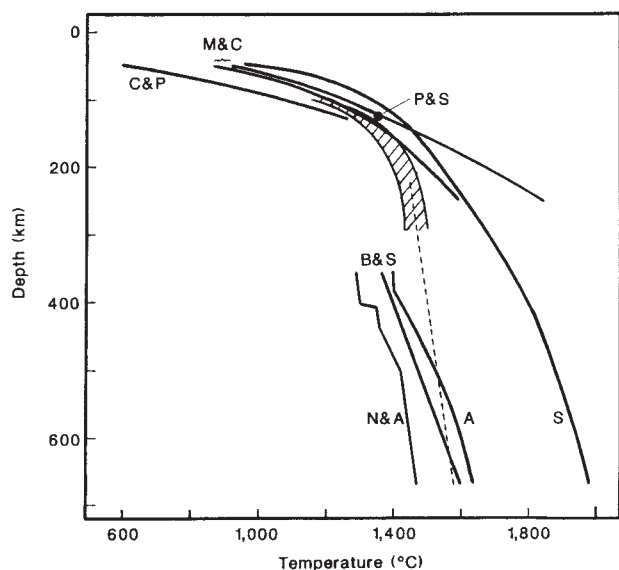


Fig. 4 Geotherms of the upper mantle. Hatched area: this study for >100 Myr; dashed line: mantle adiabat; $0.3\text{ }^{\circ}\text{C km}^{-1}$; C&P: Chapman and Pollack⁵; P&S: Parsons and Sclater⁶; M&C: Mercier and Carter²⁸; N&A: Navrotsky and Akaogi²⁹; B&S: Brown and Shankland²⁵; A: Anderson²⁶; S: Stacey²⁷.

free conditions. Because a substantial decrease in Q (Fig. 1a), and probably also in viscosity, occurs well below the onset of partial melt, mantle material seems to be greatly weakened as the temperature approaches $\sim 90\%$ of the solidus temperature. Thus, we question the conventional picture of the entire asthenosphere as a region with partial melt. Our inference is also supported by the thermal structure derived from seismic Q studied beneath the Iceland Plateau²³. Using laboratory and seismic velocity data, Sato *et al.*¹⁴ identify a small amount of partial melt ($<2\%$ volume%) in 20–50 Myr-old asthenosphere beneath the Pacific Ocean. No melt is expected in the asthenosphere older than ~ 50 Myr. The results from seismic velocity measurements¹⁴ are consistent with this study, as they suggest the absence of melt in regions older than ~ 40 Myr.

We can compare the geotherm determined in this study with that derived from other data sources. The temperature in the lithosphere has been determined from the observation of surface heat flow⁵. Reliable geotherms can be determined only in the older regions (>50 Myr), for which heat flow and temperature vary less rapidly with plate age^{5,6}. We therefore make no comparison with the results in Fig. 3a. The Q_s structure obtained in 50–100-Myr-old asthenosphere is taken to represent that at an average of 75 Myr. According to Parsons and Sclater⁶, surface heat flow is 56 mW m^{-2} at 75 Myr, which results in the geotherm indicated in Fig. 3b. For the region >100 Myr old, the seismic ray paths used by Canas and Mitchell² are appropriate for an average age of 125 Myr. Heat flow at this age is 47 mW m^{-2} (ref. 6) and gives the geotherm shown in Fig. 3c. The geotherm derived from heat flow is in good agreement with the temperature determined from seismic Q studies; the difference is $<80\text{ }^{\circ}\text{C}$. This agreement indicates that any error in temperature from the frequency dependence of Q is less than $80\text{ }^{\circ}\text{C}$. Because the laboratory measurements were made on a dry peridotite and we employ a dry peridotite solidus for determining the geotherm, this consistency suggests that oceanic upper mantle is rather dry. At greater depths, geotherms approach an adiabatic temperature gradient ($\sim 0.3\text{ }^{\circ}\text{C km}^{-1}$ (ref. 24); Fig. 3). The present results indicate a gradual change with depth in the geotherm from a conductive to an adiabatic regime.

The geotherm derived in this study is also compared, in Fig. 4, with those derived from thermodynamic parameters and equations of state^{25–27}, from heat flow and a plate model⁶, and from equilibration temperatures of mantle xenoliths²⁸ and of

the olivine-spinel transition²⁹. Our results lie within the estimates of these previous studies. Extensive seismic studies have determined Q structures of the Earth as a function of depth and plate age. But thermodynamic calculations or estimates of equilibration temperatures may not be sufficiently sensitive to derive geotherms as a function of plate age. Therefore, laboratory and seismic Q studies provide a unique means to determine both vertical and lateral temperature profiles in the Earth.

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Magma distribution across ridge-axis discontinuities on the East Pacific Rise from multichannel seismic images

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Detailed studies of the morphology of the East Pacific Rise axis have shown that its linearity is disrupted by many small but distinct non-transform offsets, including overlapping spreading centres (OSCs) and deviations from axial linearity (devals), which display variable geochemical signals^{1–9}. Using multichannel seismic reflection profiling, we have mapped the distribution of a bright, shallow reflector that Detrick *et al.*¹⁰ have associated with an axial magma chamber. We have found that it is neither continuous across the $9^{\circ}03'$ OSC⁶, nor separated into two parallel bodies², and that its lateral offset does not conform to that of the topographic offset associated with the $9^{\circ}17'$ deval. These observations provide important insight into a causative relationship between morphological and petrological segmentation in this region of the East Pacific Rise, and we speculate that the discontinuities may be the morphological response to fluctuations in the spatial pattern of magma delivery.

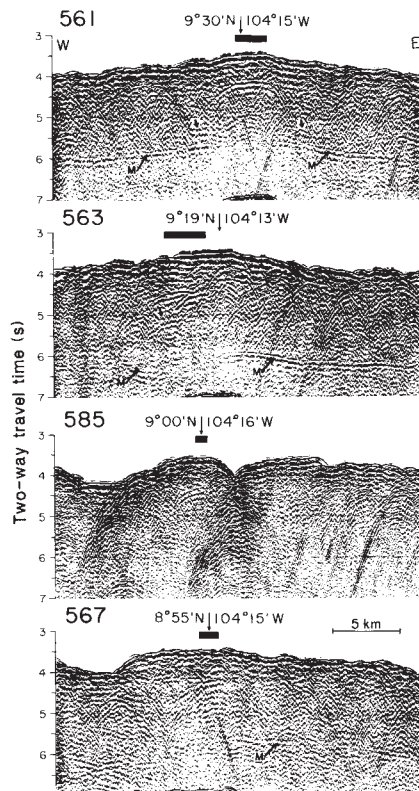


Fig. 1 Compilation of unmigrated reflection seismic profiles. Line numbers in the upper left corner refer to identified lines in Fig. 2. The vertical arrows and geographical coordinates show position of the morphologic rise axis. The strong, shallow event that Detrick *et al.*¹⁰ associate with an axial magma chamber (AMC) occurs 500–700 ms beneath the sea floor on each. The heavy horizontal bar shows the breadth of the AMC event mapped in Fig. 2, and is based on examination of unmigrated and migrated profiles. The arrow marked 'M' designates our interpretation of the Moho event; small unmarked arrows show intracrustal events.

During 1985 we conducted a series of seismic experiments on the East Pacific Rise (EPR) between 9° and 13° N, which comprised 3,500 km of single- and two-ship reflection profiling to image the substructure of the EPR, and 16 expanded spread profiles (ESPs) to provide control on the velocity structure. We obtained a unique perspective on axial structure by matching the real-time Seabeam displays with the existing Seabeam maps to shoot a line along the ridge crest throughout the region. Detrick *et al.*¹⁰ presented our initial findings and drew attention to a remarkably strong reflecting event about 600 ms beneath the sea floor along the axis, which marks the top of a low-velocity zone. This zone was recognized from analyses of coincidentally located ESPs^{11,12} at two locations. Here we present the results of a detailed mapping of the shallow reflection event in the region of two ridge axis discontinuities—the 9°17' deval and the 9°03' OSC. Note that the disruption to the ridge crest's linearity identified as the 9°17' deval actually begins at ~9°23' N, where the trend of the ridge described by the 2,600-m bathymetric contour changes from slightly west of north to more nearly north-south. The study area includes a location (at ~9°30' N) at which reflection data can be directly tied to ESP refraction results^{10,12} to show that the strong sub-axial reflector occurs at the top of a prominent low-velocity zone. This has been attributed to the presence of liquid magma in the immediate sub-axial region, that is, of an axial magma chamber (AMC)¹⁰. The shape of the magma body and its extent off-axis are not well determined. It is clear, however, that the region of melt is concentrated within a total width of ~5 km (refs. 10–12). Rela-

tively low crustal velocities exist considerably further off-axis, but are thought to be caused principally by the presence of hot rock.

A selection of unmigrated reflection profiles crossing the 9°17' deval and 9°03' OSC are presented in Fig. 1, and our mapping of the distribution of the bright sub-axial (AMC) event is shown on a Seabeam base (P. J. Fox, personal communication) in Fig. 2. Profile line 561 at 9°30' N, north of the deval, shows that sub-surface reflections are symmetrically developed about the topographic rise axis. These include the AMC event, a well developed Moho reflection and a pair of weak events in the lower crust. The latter are fairly common beneath the flanks of the rise and their origin is not well understood at present. They may represent cooling surfaces formed in the crust before it fully solidifies¹³. Symmetry of crestal morphology and crustal structure, as imaged by the reflection profiling, typifies the EPR from north of the 9°17' N deval to the Clipperton Fracture Zone.

South of this deval the AMC event broadens and a strong asymmetry is developed. The EPR axis curves in a westward direction, and line 563 shows that the AMC event is centred about 3 km west of the topographic rise crest (Fig. 1). The AMC event is also somewhat more complex; it dips westward and may actually comprise more than one event. Hale *et al.*¹⁴ previously noted an asymmetry in the magma chamber event on line 17 (Fig. 2). On line 563 the morphological rise crest is located above the extreme eastern edge of the brightest portion of the AMC event and, by inference, above the eastern flank of the magma body. Moho is well developed only on the eastern flank of the EPR. The western flank exhibits a variety of weak intracrustal events, and Moho, if present at all, appears to increase in travel time towards the rise axis. The western flank of the EPR along this segment may therefore have lower crustal velocities than the eastern flank, which could be caused by the presence of hotter crust and/or residual melt. The western flank is also somewhat shallower than the eastern ridge flank where the former is underlain by the laterally displaced AMC event (Fig. 1). At around 9°12' N the AMC event swings east towards the axis again, and then weakens considerably before terminating at ~9°03' N. Our line through the overlap basin shows that the AMC event is not present there. It appears again as a narrow discontinuous event on the western limb of the OSC at 9°02' (lines 585, 567), and by 8°50' N the AMC event occurs symmetrically beneath the broad axial high south of the OSC.

If we make an association between reflection event and the presence of magma, the observations outlined above may be used to assess the distribution of magma across the ridge axis discontinuities. Refs 10–12 have established that the occurrence of a sub-axial reflector can be reasonably associated with the presence of a magma body. Migration of the stacked data typically reduced the total width of the event by collapsing its diffracting edges, but altered its dip very little and caused minor overall position shifts. Migration also frequently introduced artefacts largely from spatial aliasing, so the relatively cleaner unmigrated profiles are displayed in Fig. 1. Migrated data were used in the mapping shown in Fig. 2. Absence of a reflecting event implies either that the magma body is actually absent or that it has shrunk to a size that is below the limit of resolution of the seismic data. On other portions of the rise axis we have observed sub-axial reflectors on as few as four stacked traces, or 200 m total width, and this probably represents the lower limit of detectability.

Given these cautionary notes, our results demonstrate that, in this area of the EPR, the morphology of the rise axis is an imperfect guide to the location of the 'axial' magma body. Consequently, the seismic data provide new insights into the relationship between the morphological segmentation of the EPR. First, although the AMC event appears to be continuous across the 9°17' deval, and is displaced in the same sense as the morphological offset, its position south of the deval is considerably west of the rise axis. Thus the magma body is apparently

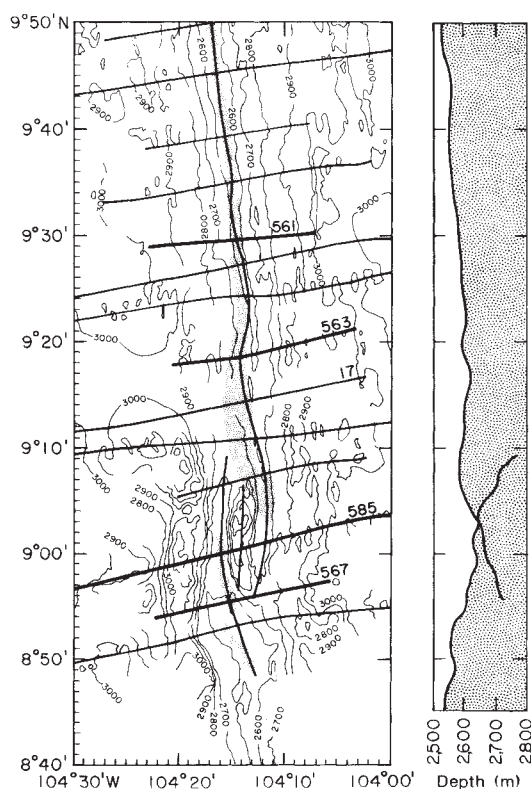


Fig. 2 Distribution of the magma body beneath the East Pacific Rise inferred from mapping the AMC event imaged in reflection profiles such as those in Fig. 1. The fourteen cross-axis reflection profiles and the along-axis profiles were used in the mapping; those with numbers are shown in Fig. 1 or referenced in the text. Seabeam bathymetry (P. J. Fox, personal communication) at 100-m contour interval is the base. The stippled area superimposed on the bathymetric chart indicates the area of occurrence of the AMC event. To the right we have shown a profile of the axial depth obtained from Seabeam mapping by Macdonald and Fox¹⁹. Note that the axial seismic lines do not always correspond to the shallowest point on the rise axis.

continuous across the deval but has suffered a substantial westward deflection which could act to restrict the lateral communication of magmas across the deval. North of the deval, any magma erupted at the ridge crest would probably be fed from the centre of the magma body, whereas south of the deval erupted lavas would be fed from the extreme eastern edge of the magma body. Factors such as these may in part account for some of the observed petrological discontinuities³ at small ridge crest offsets.

Second, because there is no location at which a resolvable AMC event continues across, or lies adjacently beneath, both limbs of the 9°03' N OSC, we infer that there is no significant communication of magmas across the OSC and that a geochemical discontinuity may therefore be expected³. Along much of the overlapping limbs, magmas must either be transported laterally up to 5 km ahead of their associated magma bodies, or be injected vertically from small, transient magma bodies². Evolved FeTi basalts, reflecting high degrees of fractionation, have been recovered from the tips of OSC limbs^{2,3,5}. The magma bodies resolved by the reflection profiling do not actually overlap at the OSC, and their distribution does not suggest that either limb is more robust than the other.

Although the time sequence of development of devals and OSCs is largely speculative, it is generally considered that they are dynamic features of ridge morphology^{2,4,7,15-18}. The lack of spatial coincidence between morphological rise axis and magma supply suggests a degree of temporal decoupling also. If we suppose that it is energetically favourable for the main region

of magma eruption to be located centrally above the magma supply, devals and OSCs may represent attempts by the ridge to reposition in response to shifts in magma supply. We speculate that these small physiographic segment boundaries may result from, rather than control, changes in the magma delivery system.

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Mammal teeth from the Cretaceous of Africa

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We report here the discovery of two mammal teeth from the early Cretaceous of Cameroon. These, and some jaw fragments, all from Cameroon, are the only fossil evidence of mammalian evolution from Africa between late Jurassic and Paleocene, a span of at least 85 million years. A triangular upper tooth lacks the principal internal cusp of marsupials and placentals and is therefore of a similar evolutionary grade to most Jurassic and early Cretaceous therian mammals, but more primitive than the metatherian-eutherian grade. Early Cretaceous, or older, therian mammals are now known from all southern continents except Antarctica. The new find from Cameroon is consistent with the hypothesis that marsupials, the dominant living mammals of South America and Australia, were not present on any Gondwana continents until after the early Cretaceous separation of Africa by the opening of the South Atlantic.

Two mammalian teeth were recovered by sieving seven metric tons of sediment from locality KB-6 in the Koum Basin (Fig. 1). In addition to the mammalian remains, pollen (*Classopollis* sp.) and a diverse vertebrate assemblage were recovered. Verte-

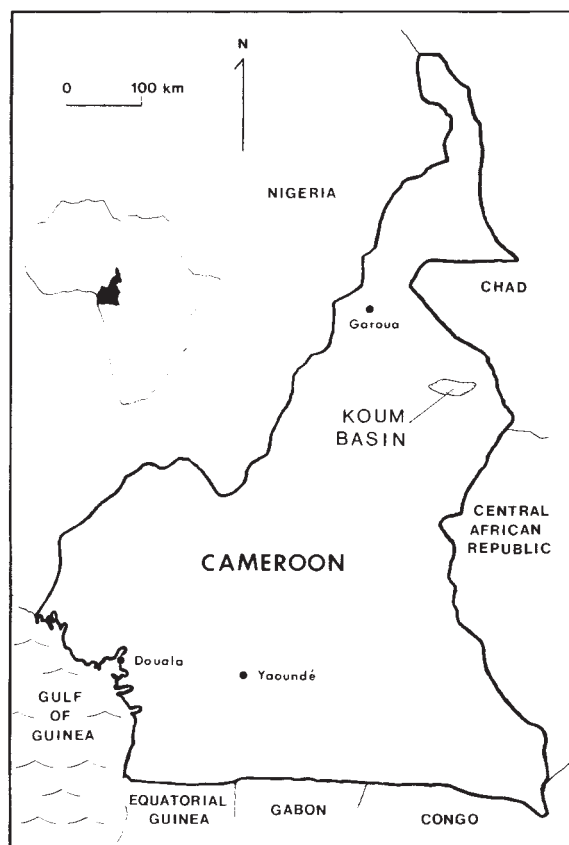


Fig. 1 Location map of the Koum Basin.

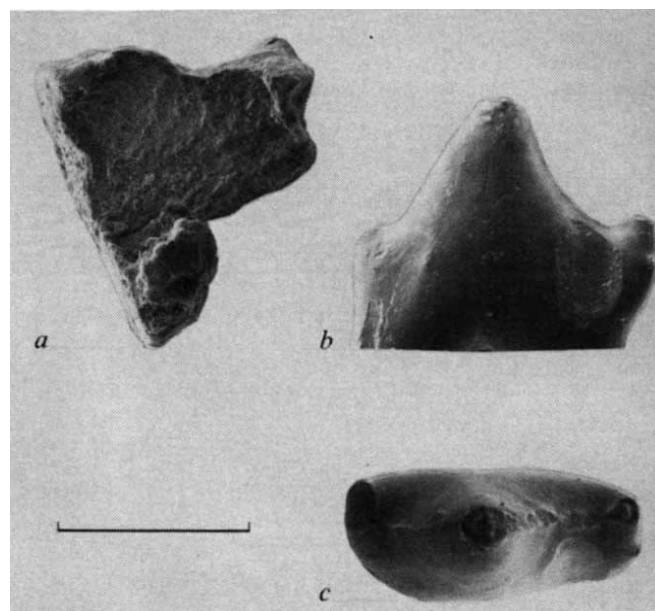


Fig. 2 Mammal teeth from the Lower Cretaceous of Cameroon. *a*, occlusal view of upper right molar (CAM-54); lingual cusp at bottom, notch for interlocking with adjacent tooth in upper right (anterior) angle. *b-c*, Left lower premolar (CAM-31): *b*, labial view; *c*, occlusal view. Scale bar equals 1 mm.

brates include fish (semionotiform and dipnoan), frogs, turtle, lizards, *Araripesuchus* and other crocodylians and theropod, sauropod and iguanodont dinosaurs. Fossils were preserved in fluvial sediments on which a paleosol was subsequently developed¹.

The Koum Basin is part of a structural complex related to the opening of the South Atlantic and the separation of Africa from South America. This occurred during the latter half of the early Cretaceous². The fauna from KB-6 is similar to that of the better known Gadoufaoua fauna of Niger, especially in the presence of *Araripesuchus* and iguanodonts. The age of Gadoufaoua is tentatively considered to be Aptian³, with the upper age limit set by overlying marine strata of Cenomanian age. The lower age limit of Gadoufaoua is Barremian, on the basis of lithostratigraphic correlation³. It is therefore likely that KB-6 is late early Cretaceous.

The triangular occlusal outline of the mammalian upper tooth (CAM-54, Fig. 2) is clearly therian but differs from marsupials, placentals, and other mammals of metatherian-eutherian grade. The tooth (length = 1.48 mm, width = 1.60 mm, lingual angle = 48°), preserved in a maxilla fragment, is corroded. Two of the three sides are well defined by enamel and the third side is partially complete. The labial margin is sinuous and indented between anterior and posterior portions. The anterior apex bears a distinct notch for interlocking with an adjacent tooth. There is no indication of a metacone or of cingula. The specimen has a high lingual cusp as indicated by remaining enamel, contrasting with the low protocone of early Cretaceous mammals of metatherian-eutherian grade^{4,5}, suggesting that the cusp is not a protocone but a paracone. So far as can be determined, the specimen is most similar to cladotheres (but not Tribosphenida) in that the protocone is absent, the angle of the tooth is less than 100° and the styler region is interlocking⁶.

The lower tooth (CAM-31; Fig. 2; length = 1.68 mm, width = 0.72 mm) is double-rooted and has three cusps arranged

anteroposteriorly, with a smaller cusp in the posterolabial portion. There is a strong, striated wear facet on the posterolabial side of the tooth and a less pronounced anterolabial wear facet. Abrasion is apparent on the crest connecting the cusps, as well as on the tips of the cusps themselves. The lower tooth is unlike triconodonts that have more prominent anterior and posterior cusps. The tooth is asymmetrical and lacks accessory cusps and cingula, characteristics that are consistent with assignment to Theria. The specimen, if therian, was probably situated near the back of the pre-molar series judging from the anterior and posterior cusps. The two Cameroonian teeth are of comparable size, but no conclusions can be drawn as to whether they are conspecific.

Assignments of the upper tooth to the Cladotheria is generally consistent with Bonaparte's hypothesis for vertebrate evolution in the Cretaceous of southern continents⁷. He suggested, based mainly on the South American record, that dryolestid mammals (a family of primitive therians) underwent vicariant evolution in Gondwana continents after the breakup of Pangea^{7,8}. Other than the early Jurassic morganucodontids *Megazostrodon rudnerae* and *Erythrotherium parringtoni*, the only previously known Mesozoic mammal from Africa is *Brancatherulum tendagurensis*, an edentulous jaw from the late Jurassic of Tanzania. The jaw of *Brancatherulum* is advanced over morganucodontids because it has a true angular process⁹ and no pseudangular process¹⁰. *Brancatherulum*, the upper tooth from Cameroon, and dryolestids are all Cladotheria⁶ more primitive than the metatherian-eutherian grade, which is known from the early to mid-Cretaceous of northern continents. Although the fossil record of Mesozoic mammals on southern continents is woefully inadequate, it is clear that mammals were widely distributed across all continents before the origin of marsupials and placentals^{11,12,13}. The fossil record, such as it is, documents a diversification of primitive mammals on southern continents before the first record of marsupials, which now dominate the mammalian faunas of Australia and South America^{14,15}.

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Individual optimization of clutch size in great tits

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Individual birds within a population often lay clutches of very different sizes^{1,2}, even though those laying the larger clutches tend to produce more young which survive to enter the subsequent breeding population (recruits)³. Two hypotheses have been proposed to account for such differences in clutch size⁴. The individual optimization hypothesis proposes that parents lay that size of clutch from which they can maximize recruitment: adding or taking away young from their nests will result in lowered recruitment⁵. The trade-off hypothesis assumes a cost of reproduction; the rearing of offspring leads to the parents having a lowered future survival or fecundity^{6,7}. When given experimentally enlarged broods to rear parents should therefore show an increased mortality or reduced fecundity. We tested the predictions of these two hypotheses by manipulating the brood size of great tits *Parus major* over nine years, and following subsequent survival of offspring and parents and future breeding performance of parents. Parents differed in their ability to recruit offspring, this being reflected in the size of clutch that they laid. Parents did best, in terms of the number of offspring recruited, by rearing their own clutch size: adding or removing young did not increase recruitment rates. These results strongly support the individual optimization hypothesis. There was no evidence that parents raising enlarged broods suffered a higher mortality or decreased fecundity compared with those which raised their own natural or decreased broods. We are therefore unable to establish a cost of reproduction and consequently the predictions of the trade-off hypothesis are not supported.

Individual birds differ widely from one another in the number of eggs which they lay. Each year great tits (*Parus major*) nesting in Wytham Woods, Oxford, commonly lay clutches varying from 5 to 13 eggs, even though the pairs raising large broods usually raise more recruits (young which survive to breed in Wytham). Two hypotheses have been proposed to account for this apparent anomaly, both centring on the idea that the numerical benefit of having a large number of offspring must be offset either by the reduced viability of each individual offspring (the individual optimization hypothesis) or by the future reproductive potential of the parents (the trade-off hypothesis).

The individual optimization hypothesis, derived from

Lack's^{1,8} ideas by Perrins and Moss⁵, states that individuals lay that number of eggs which will maximize the number of recruits produced from a single season; individual differences in the ability to rear offspring mean that the optimum clutch differs between individuals. A number of studies, however, seem to show that individuals would raise more recruits if they laid slightly more eggs than they actually do. This could be explained by the trade-off hypothesis, if raising the within-season optimum brood resulted in reduced future breeding output due either to reduced parental survival or fecundity (or both)^{6,7}.

To decide how well the two hypotheses account for clutch-size variability it is essential to carry out brood manipulations. To take account of differential post-fledging mortality it is necessary to measure offspring recruitment rather than simply the number of fledglings and to measure survival rates of parents between years. Specifically, the individual optimization hypothesis predicts that offspring recruitment is highest from those nests where all the eggs laid are raised (control nests): subtraction of or addition of young to broods should reduce the number of recruits from these nests. The trade-off hypothesis predicts that adding young may increase the number of recruits in a season, but will lead to a reduction in the subsequent survival or fecundity of the parents.

We have tested both these hypotheses. During the years 1959–1963 and 1977–1980 inclusive, the brood size of great tits nesting at Wytham was manipulated, usually by adding or removing 3–4 young just after hatching ($N = 483$ nests). In addition, 784 nests which were not manipulated were also available for analysis. Parents did not discriminate between their own and foster young. Four classes of nests could therefore be identified: (1) those where brood size differed from clutch size due to natural losses ($N = 449$), (2) those where brood size differed from clutch size through removal or addition of young ($N = 227$), (3) those where brood size differed from clutch size as a result of both natural losses and manipulation ($N = 260$), and (4) those where brood size equalled clutch size (control nests) ($N = 331$). A generalized linear model, fitted using GLIM⁹, was not significantly altered by entering natural losses and manipulations as separate categories, so we have lumped these in our analyses, using only the difference between clutch size and subsequent brood size as the manipulation score.

Figure 1 shows number of recruited young in relation to manipulation and clutch size, estimated from the statistical model:

$$\log \text{number recruited young} = \log \text{number nests} + ax_1 + bx_2^2 + cx_2$$

where x_1 is original clutch size and x_2 is manipulation and a , b and c are fitted parameters. We used a logarithmic link function with Poisson errors because of the strongly positively skewed distribution for the number of recruits per nest, the majority of nests producing no recruits at all.

Table 1 log-linear model using maximum likelihood estimates fitted to the number of great tit offspring recruited per nest

Model	Deviance	d.f.	Change in deviance	P
Null model	312.02	91		
+ clutch size	211.81	90	–100.21	<0.0005
+ (MANIP.) ²	127.15	89	–84.66	<0.0005
+ MANIP.	117.38	88	–9.77	<0.0050

Clutch size, manipulation and manipulation squared all significantly reduce the deviance of the fitted null model. (ΔD approximates the χ^2 distribution with respective d.f.). The number of offspring recruited per nest follows a Poisson distribution, and this is entered via the error term, resulting in a log linear plot through a log link function, with the offset defined by the log number of nests in each cell. Scale parameter = 1.334.

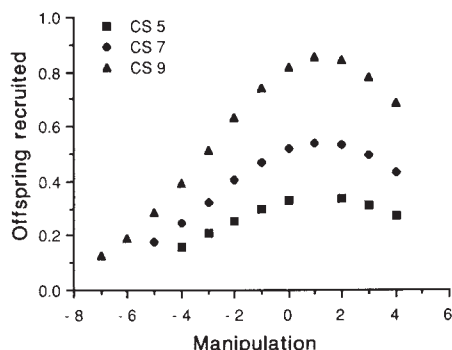


Fig. 1 The average number of offspring recruited per nest estimated from the log-linear model described in the text, plotted against manipulation score and separated with respect to clutch size. For clarity only three original clutch sizes have been shown: those not illustrated follow the same pattern. Note how recruitment is maximized at or close to zero manipulation.

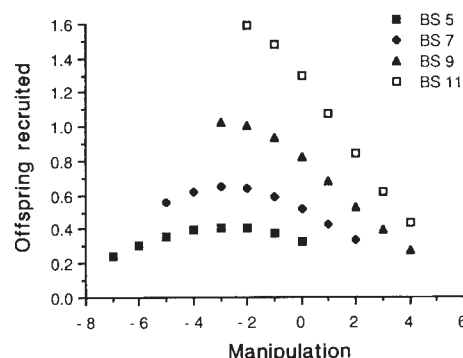


Fig. 2 The number of offspring recruited as in Fig. 1, but this time grouped according to final brood size. Note that, except for the more extreme removal nests, that broods which originally had larger clutches tended to result in more offspring being recruited for any given final brood size.

Clutch size, manipulation and its square all significantly reduced the deviance of the null model, indicating their relevance in predicting the number of offspring recruited (Table 1). After removing year effects from the model, the above three variables still all significantly reduced the deviance of the null model. As expected, birds laying more eggs do consistently better regardless of manipulations. The maximum number of recruits are produced at a manipulation value of ~ 1.21 , where 0.006 additional young per nest are recruited compared with control nests (Fig. 1). However, recruitment is known to be negatively correlated with date of hatch^{10,11}; the average decrease in offspring survival with increasing hatch date for the great tits at Wytham for 1977 to 1980 was 0.028 young per day. Thus parents which laid one extra egg would lose more from the consequent delay in hatching than they would gain from having an extra chick. Therefore, from these analyses we conclude that parent great tits are maximizing the number of young they recruit by rearing a brood equal or very similar in size to the clutch they originally laid.

It follows that, within each brood size, the most successful nests will be those which started with the largest clutch size. This can be seen in Fig. 2, where the estimated recruitment rates are plotted against manipulation for different resultant brood sizes (original clutch size plus manipulation). This procedure was adopted, rather than re-fitting using resultant brood size as an independent variable, because of the strong collinearity between manipulation and brood size; larger resultant broods are more likely to result from additions and smaller broods from subtractions. Taking broods of nine, for example, those birds which started with 11 eggs (manipulation = -2) are more successful than those which started with 10 (= -1) which are in turn more successful than those which started with 9(0) and so on. This must arise from intrinsic or extrinsic differences between individuals, the differences in 'ability' or 'quality' of the parents being reflected in the number of eggs that they lay. This provides convincing support for the individual optimization hypothesis.

Figure 2 also shows that parents experiencing large negative manipulations tend to show reduced success. For example, birds reduced to five young from an initial ten produce less recruits than those laying five to start off with. This suggests that they are aware of large scale losses and may not work so hard, or may be more likely to desert the nest altogether.

We carried out two analyses of survival in relation to manipulation to test the trade-off hypothesis. We used GLIM to carry out a logistic regression of clutch size and manipulation on the probability of parental survival. For females ($N = 955$) neither clutch size ($\Delta D = 2.2$; 1 d.f.; NS) nor manipulation ($\Delta D = 0.20$; 1 d.f.; NS) affected their survival. Adding a factor for year showed that different years had different parental survival rates ($\Delta D = 35.7$; 7 d.f.; $P < 0.001$). The year $\times$ manipulation interac-

tion was not significant ($\Delta D = 13.2$; 7 d.f.; NS). Analysing separately for brood size gave similar results, again contrary to the trade-off hypothesis. A similar analysis of male survival (restricted to the years 1977-1980 because males were not systematically caught in the earlier years) again showed no significant effects on mortality of clutch size, manipulation or brood size. Therefore, there is no evidence of a survival cost of reproduction in either male or female great tits.

For those birds which survived to breed in the season following the manipulations we compared clutch size, number fledged and number of recruits to see whether there was any effect of raising extra young on future fecundity. Neither the clutch size in the following season ($F = 0.69$; 2,374 d.f.; NS) nor the number fledged ($F = 0.44$; 2,280 d.f.; NS) was affected by manipulations. To investigate effects on number of recruits we used a model with Poisson errors and log link as before. After fitting a factor for year ($\Delta D = 66.9$; 7 d.f.; $P < 0.001$; differences in recruitment rate between years are highly significant) the effect of manipulation the previous year was not significant ($\Delta D = 4.61$; 2 d.f.; NS). The same analysis for surviving males, restricted to 1977-1980 as before, gave similar results. Though the sample sizes are small, particularly for survivors who had had positive manipulations, we conclude that there is no evidence for a loss in future fecundity caused by raising additional young.

These results demonstrate unambiguously that the "optimal clutch size is n for birds that choose to lay n eggs"¹² convincingly supporting the individual optimization hypothesis. We were unable to find a cost of reproduction affecting either parental survival or fecundity through to the following breeding season. We therefore conclude that (1) individual birds are laying that size of clutch from which the largest number of recruits are likely to survive, (2) the clutch size laid by an individual is an accurate reflection of its ability to raise young which survive to breed: adding or removing young reduces rather than increases the number of their offspring which parents subsequently recruit, and (3) there is no evidence for a survival cost of reproduction either in respect of brood size or manipulation. These results are entirely consistent with the individual optimization hypothesis, but contrary to the trade-off hypothesis.

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Direction of self-motion is perceived from optical flow

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Can moving observers distinguish the direction in which they are moving from the direction in which they are looking? Radial patterns of optical flow could be used to perceive the translational direction of self-motion and to guide locomotion. However, these patterns are confounded by eye movements, which has led previous investigators to reject the outflow pattern and to propose alternative strategies. Using computer-generated displays of optical flow, we show here that humans can perceive their direction of self-motion during stationary fixations, pursuit eye movements, and with displays that simulate the effects of eye movements. We conclude that optical flow is sufficient for perceiving the direction of self-motion and provide evidence for a theory based on differential element motion.

Gibson¹ was the first to show that translation of an observer through a stationary environment produces a radial pattern of optical flow at the eye (Fig. 1). The fixed point or 'singularity' in the flow field, called the focus of outflow, specifies the observer's direction of self-motion, or 'heading'. We have found that humans can perceive their direction of self-motion from such optical outflow patterns with accuracies of 1° of visual angle². However, the retinal flow field is radically altered by observer rotation, such as a pursuit eye movement to track a point on the passing ground surface. This adds a constant displacement to the flow pattern, replacing the focus of outflow at the destination point with a new singularity at the fixation point (Fig. 2).

A number of hypotheses have been proposed to explain how, in this case, the direction of self-motion could be perceived. (1) Gibson^{1,3} suggested that the effects of observer translation and rotation could be decomposed visually, but did not demonstrate this formally. The problem is not trivial, and it is only recently that some general computational solutions have been derived⁴⁻⁷. A less general decomposition could be based on differential motion between neighbouring elements, because the difference vectors radiate approximately from the destination point⁸, but this requires elements at different depths within some neighbourhood. (2) Motion parallax at depth edges is due solely to observer translation and thus could be used to eliminate the rotational component⁴, but this solution requires edges. (3) The local maximum of divergence in the velocity field is invariant under rotation; this could be used⁹ but it seldom corresponds to the direction of heading and fails in complex environments². (4) Asymmetries in the flow pattern could guide a sequence of fixations to the focus of outflow¹⁰⁻¹², but this requires multiple fixations. (5) Finally, an extra-retinal oculomotor signal, such as an efference copy, could be used to compensate for eye movements¹³. Regan and Beverley¹⁴ reported poor performance with simulated rotation during translation toward a plane, but we will show this is a special case. We now present evidence that observers can perceive their direction of heading during an eye rotation and that Gibson's original approach was on the right track.

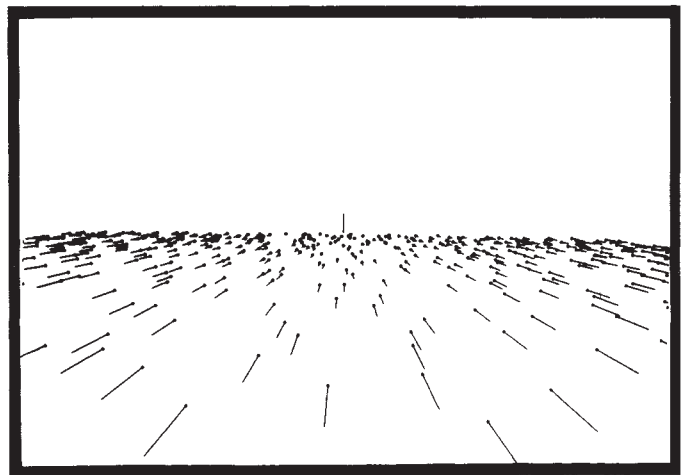


Fig. 1 Optical velocity field produced by observer translation parallel to the ground plane. Vertical line indicates the focus of outflow, a singularity that specifies the direction of self-motion.

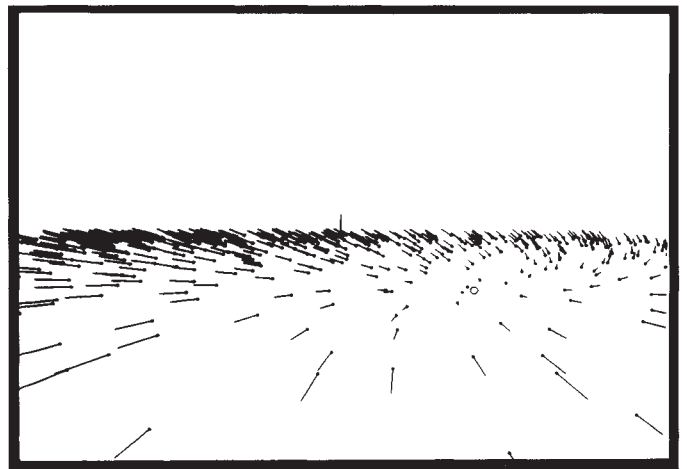


Fig. 2 Retinal velocity field produced by combined observer translation and rotation, due to translating toward vertical line while fixating circle on ground surface. The singularity is now at the fixation point, not the destination point.

In the first experiment we compared heading judgements during stationary fixations and pursuit eye movements, testing the necessity of edge parallax and multiple fixations. We presented computer displays of observer translation parallel to a random-dot ground plane with no depth edges. In the last frame of each display, motion stopped and a vertical target line appeared on the horizon; the subject had to judge whether he or she seemed to be heading to the left or right of the target. In half the trials, the subject looked at a stationary fixation point on the screen, so that the retinal flow field only contained a translational component (Fig. 1). In the other half, the fixation point moved as though it were an object on the ground surface and induced a pursuit eye movement, so that the retinal flow field contained both translational and rotational components. Eye movements were monitored with a head-mounted limbus tracker accurate to $\pm 0.5^\circ$ and the output was superimposed on a videotaped image of the stimulus display. Trials containing sequences of fixations or drifts of more than 1° from the fixation point were excluded from further analysis; fewer than 5% of trials were rejected.

The results show accurate heading judgements (Fig. 3), with the overall mean percentage correct significantly above that expected by chance under both conditions (stationary, $t(7) = 19.43$, $P < 0.001$; moving, $t(7) = 14.65$, $P < 0.001$) and no statis-

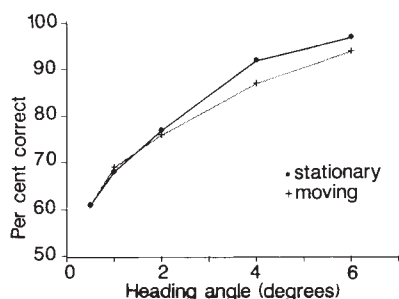


Fig. 3 Results of first experiment with stationary and moving fixation point, mean percentage correct for eight observers. Displays corresponded to fast walking speed of 1.9 m s^{-1} and dot density of 0.12 dots m^{-2} on the ground surface, assuming an eye height of 1.6 m , with 63 dots visible at the start of each trial. Screen subtended 40° horizontal $\times$ 32° vertical viewed binocularly and eye movements were monitored with a binocular limbus tracker. The target position varied between $\pm 1.0^\circ$ and 3.0° from the centre of screen; the heading angle with respect to the target varied from $\pm 0.5^\circ$ to 6.0° ; display duration was 3.7 s . The fixation point appeared in a random position on the ground so that it would remain on the screen; total excursion varied from 2° to 10° , mean velocity from 0.5 to 2.7° s^{-1} .

tical difference between them ($t(7) = 1.63$, NS). Mean thresholds of 75% correct were at heading angles of 1.7° under stationary conditions and 1.8° under moving conditions ($t(7) = 1.96$, NS). Thus, observers can accurately perceive the direction of self-motion during pursuit eye movements, separating the effects of translation and rotation without edge parallax or multiple fixations.

To test the hypotheses about oculomotor signals and visual decomposition, we placed optical flow in conflict with oculomotor signals in a second experiment. In case the visible edges of the display screen provide a rotation-invariant frame of reference for the radial outflow pattern, they were minimized by a translucent reduction screen. In half the trials, we presented displays with a moving fixation point that the observer tracked, as before. In the other half, matched displays simulated the retinal flow pattern that would occur with such an eye movement, but the fixation point was actually stationary on the screen (Fig. 2). Thus, both the oculomotor state and any frame effects indicated that no eye rotation was occurring, whereas the optical flow pattern specified translation plus eye rotation. If subjects saw themselves heading toward the singularity at the fixation point, the performance level would be that expected by chance.

The results again show accurate heading judgements (Fig. 4) with the mean percentage correct significantly above the chance level in both conditions (moving, $t(5) = 10.90$, $P < 0.001$; simulated, $t(5) = 11.79$, $P < 0.001$). There are marginally significant differences between conditions at heading angles of 2° ($t(5) = 3.46$, $P < 0.05$) and 3° ($t(5) = 3.16$, $P < 0.05$), possibly due to frame effects. But there was no difference between the mean thresholds of 1.3° in the moving condition and 1.5° in the simulated condition ($t(5) = 1.79$, NS).

Interestingly, observers generally could not distinguish moving from simulated trials; in the latter there was a strong illusion that one's eye was actually moving. It is possible that the radial flow pattern was detected during the initial latency before pursuit in the moving condition, but this could not account for the accuracy observed in the simulated condition because no radial pattern was ever present during simulated displays. Thus, an oculomotor signal is not necessary to separate translation from rotation, consistent with Gibson's visual decomposition theory.

To test the necessity of a divergence maximum, we repeated this experiment with movement through a three-dimensional

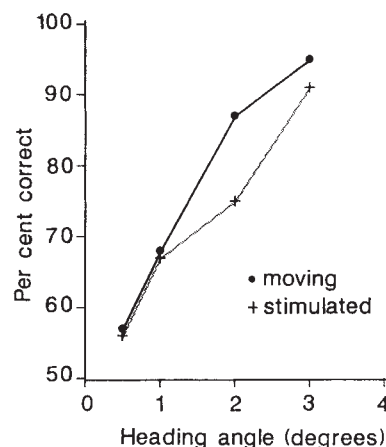


Fig. 4 Results of second experiment with moving fixation point and simulated eye movement, mean percentage correct for six observers. Displays were viewed monocularly through translucent reduction screen that eliminated luminance differences at the edges of the display, so eye movements were not monitored. The heading angle varied from $\pm 0.5^\circ$ to 3.0° , display duration was 3 s . The fixation point appeared in a random position on the ground within 5° of the target; total excursion varied from 1° to 2° , mean velocity from 0.3 to 0.7° s^{-1} .

cloud of dots. No single maximum is present in such a cloud and other differential invariants^{7,9}, based on spatial derivatives that assume a continuous flow field, are undefined. Once again, subjects performed with equal accuracy under both conditions, with mean thresholds of 1.2° in the moving condition and 1.4° in the simulated condition ($t(3) = 1.78$, NS). This provides confirmation of our previous conclusion² that observers do not rely on the maximum of divergence or related differential invariants.

In the special case of movement perpendicular to a plane, however, we found that performance was at chance levels in the simulated condition (see also ref. 14). Here, the singularity at the fixation point is the centre of a radial outflow pattern, as in the case of pure translation toward the fixation point, and indeed subjects see themselves as heading toward the fixation point. This is not in agreement with general models of the visual decomposition⁴⁻⁷ and indicates that differential motion produced by elements at different depths is necessary.

These results show that observers can distinguish where they are heading from where they are looking on the basis of optical flow alone, without oculomotor signals, multiple fixations, edge parallax, or differential invariants, and they contradict general models of decomposition. They are consistent, however, with models based on differential element motion⁸, for which there is some neurophysiological evidence¹⁵.

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Localization of a susceptibility locus for schizophrenia on chromosome 5

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Schizophrenia is a common disorder with a life time prevalence of approximately 1 per cent¹. The illness often develops in young adults, who were previously normal, and is characterized by a constellation of symptoms including hallucinations and delusions (psychotic symptoms) and symptoms such as severely inappropriate emotional responses, a disorder of thinking and concentration, erratic behaviour as well as social and occupational deterioration². A considerable proportion of the variance in the liability to develop schizophrenia may be genetic^{3,4}, but segregation analysis, to establish a mode of transmission, has not produced a consistent result⁵⁻¹⁹. One of these studies was carried out in Iceland and made use of the large family size and extensive genealogical information present in that country^{16,17}. Here we demonstrate genetic linkage of two DNA polymorphisms on the long arm of human chromosome 5 to schizophrenia in seven British and Icelandic families with multiple affected members. The results indicate the existence of a gene locus with a dominant schizophrenia-susceptibility allele. Inheritance of the allele in the families studied suggests that it may also predispose to psychiatric conditions such as schizophrenia spectrum disorders and a variety of other disorders. This report provides the first strong evidence for the involvement of a single gene in the causation of schizophrenia.

Linkage studies with genetic markers provide a much more powerful method of establishing genetic aetiology in humans than methods such as segregation and path analysis. Segregation analysis tests whether the total proportions of affected individuals in a family fit Mendelian (single-locus) expectations. Linkage analysis tests the co-segregation of disease and linkage marker in each individual meiosis in a family. Previous linkage studies to localize susceptibility alleles for schizophrenia to specific chromosomes have been negative²⁰⁻²⁴. Our strategy for genetic research into schizophrenia was to use highly polymorphic 'minisatellite' restriction fragment length polymorphisms (RFLPs)²⁴ for linkage analysis and to do linkage studies at loci previously implicated by cytogenetic abnormalities localized in schizophrenics^{25,26}. A recent report of a chromosomal abnormality in a Chinese man and his nephew both suffering from schizophrenia, facial dysmorphism and other abnormalities implicated chromosome 5q11-13 (ref. 26). This evidence, although weak, gave us the impetus to study the chromosome 5 region in question by using linkage markers. Two preliminary studies of chromosome 5 using an RFLP at the lymphocyte glucocorticoid receptor gene locus²⁷ localized to 5q11-5q13 gave weakly positive linkage to schizophrenia^{28,29}.

To investigate linkage to this region of chromosome 5 we studied five Icelandic and two British kindreds with multiple cases of schizophrenia in at least three generations. The five Icelandic families were ascertained after identification of schizophrenic probands who were amongst currently hospitalized

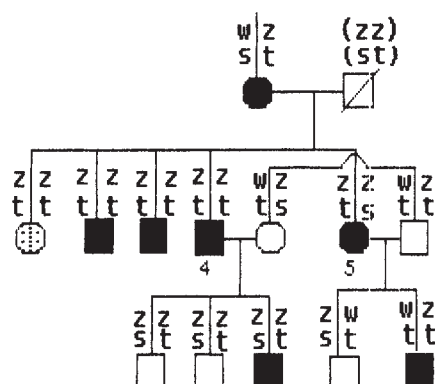
patients during 1986 and 1987. The two British families were selected on the basis of having four or more affected individuals. Extensive genealogical tracing was performed to ensure, as best we could, that there was a single unilateral source for any possible dominant disease allele entering into each kindred.

In the seven pedigrees there were 104 individuals who were still alive and who had descendants or ancestors who had been diagnosed as schizophrenic. All 104 people were personally interviewed by a psychiatrist for the study. 98 of these received a full interview using the Schizophrenia and Affective Disorders Schedule—lifetime version (SADS-L)³⁰ and the remaining six unaffected individuals were screened more briefly for the absence of a psychiatric disorder. The SADS-L information obtained at interview, and extensive medical information made available to us from other sources, were used to classify all cases according to both the Research Diagnostic Criteria (RDC)³⁰ and some of the clinical categories of the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSMIII) (ref. 31). DSMIII and RDC diagnoses are the two most widely used systems of psychiatric diagnosis for research purposes and have been shown to be reliable and valid. Independent validation of diagnosis from all sources of information was carried out by two further psychiatrists. This consisted of reviewing all the available information on individuals who received any psychiatric diagnosis and, in the event of uncertainty, more information was obtained until a consensus was reached. Psychiatric interviewing and diagnosis was carried out blind to the results of linkage work in the laboratory. The results are described here.

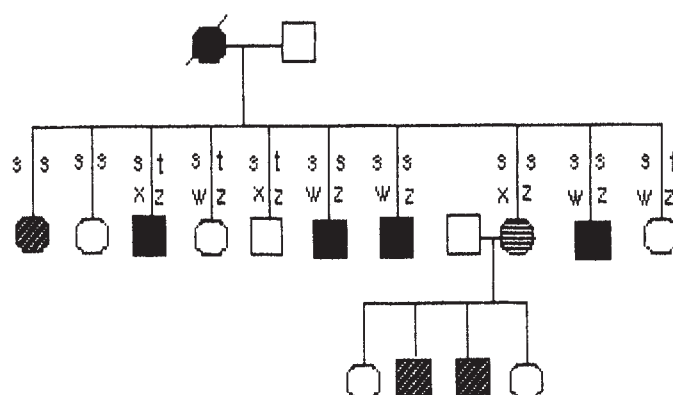
(1) Amongst the 104 individuals there were 39 cases of schizophrenia as defined by the RDC. Under the slightly more stringent DSMIII criteria 31 of these qualified as schizophrenic. The remaining eight were classified as having schizophreniform disorder (DSMIII) or unspecified functional psychosis (RDC) and were included as cases of schizophrenia. The patients exhibited a wide variety of the clinical subtypes of schizophrenia (paranoid, hebephrenic and so on) and all had histories of psychiatric hospitalization and often prolonged treatment with antipsychotic drugs. (2) A further five individuals were diagnosed as having schizoid personality disorders (DSMIII) with ($N=2$) or without ($N=3$) schizotypal features as defined by the RDC. We refer to this group as those with schizophrenic spectrum disorders. (3) A further ten individuals had psychiatric illnesses which were not part of the schizophrenic or schizophrenic spectrum disorders. Some of these individuals had received continuous periods of treatment with anti-psychotic drugs even though they had not been diagnosed as schizophrenic. We refer to these cases as 'fringe' phenotypes (Fig. 1).

Fig. 1. Seven multiplex families gave informed consent and provided blood samples for DNA extraction. Paternity was confirmed using a combination of blood group markers or minisatellite DNA fingerprints²⁴. DNA from blood samples was digested with *MspI*, *XbaI* and *TaqI* restriction enzymes. Digested DNA was separated by size in 0.8% agarose gels and transferred by Southern blotting to nylon filters according to standard techniques³⁰. Probes p105-599Ha and p105-153Ra were labelled with ³²P and hybridized to the filters. p105-599Ha reveals a three-allele system with *TaqI* giving allelic fragments 17 kilobase (kb), 14 kb and 10 kb in size shown as W, X and Z (allele frequencies 0.32, 0.16 and 0.52 respectively). Probe p105-153Ra hybridizes to *XbaI* allelic fragments that are 8.7 kb and 5.8 kb (shown as s and t) with allele frequencies of 0.33 and 0.67. p105-153Ra/*MspI* RFLPs (not shown or included in the analysis) were found to be in linkage disequilibrium with *XbaI* fragments and were 6.3 kb and 5.2 kb in size with allele frequencies of 0.29 and 0.71. RDC diagnosis is shown in the pedigree diagrams according to the key. The sex of individuals has been altered to maintain confidentiality. The kindreds shown are part of larger pedigrees containing other unsampled affected cases. The structure of one pedigree has been changed slightly to protect confidentiality. The change has no effect on the lod score.

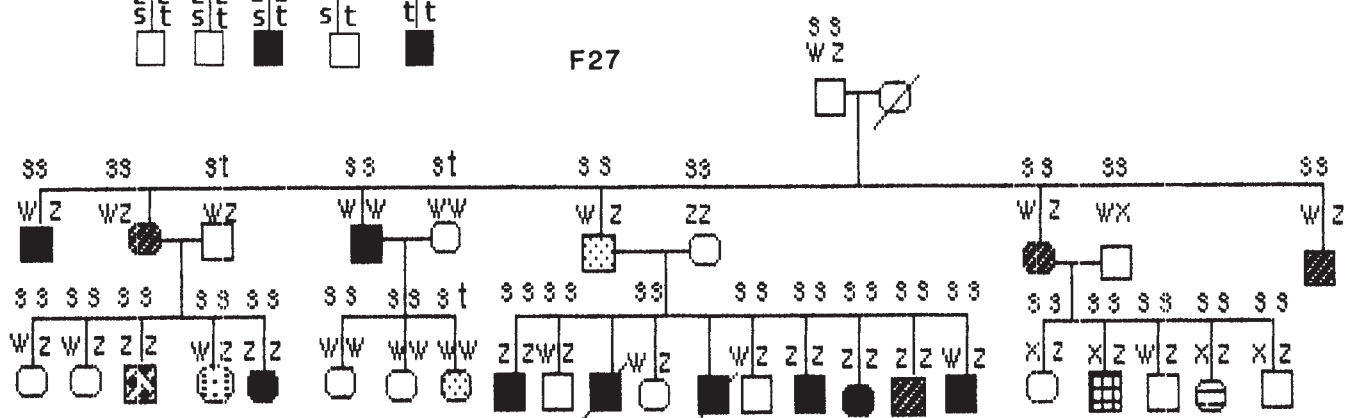
F20



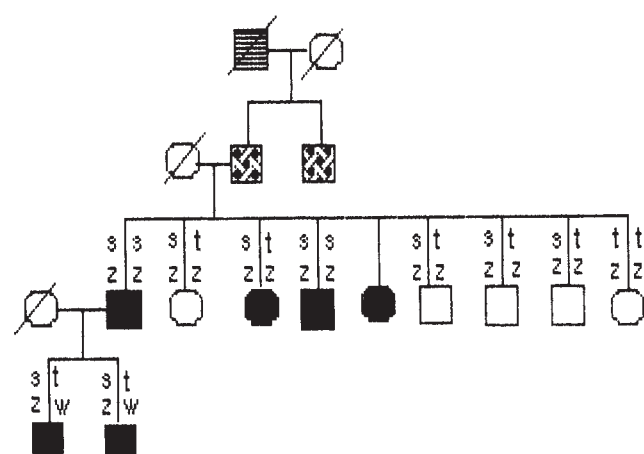
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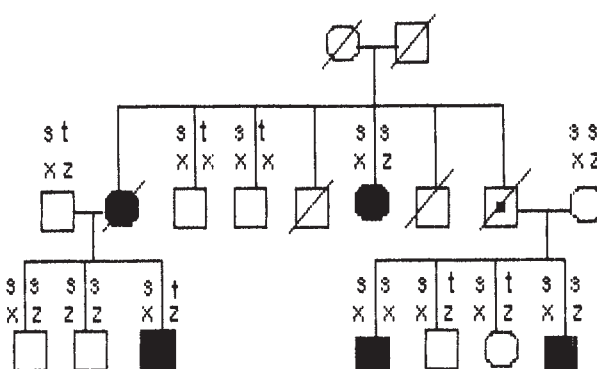
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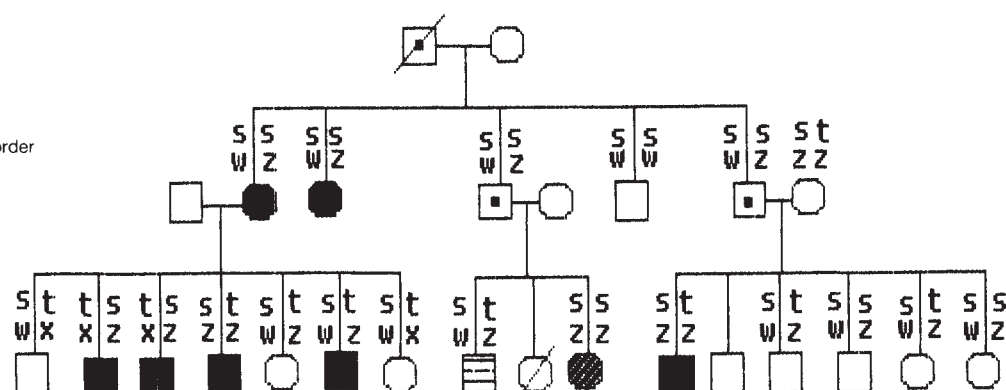
F40



F74



F41



- Unaffected, deceased
- Normal obligate carrier
- Schizophrenia, schizoid personality disorder or unspecified functional psychosis
- Schizoid personality disorder with or without schizotypal features
- Major depressive disorder
- Minor depressive disorder
- ▨ Alcoholism
- ▤ Phobic disorder
- ▦ Drug use disorder
- ▧ Other psychiatric disorder

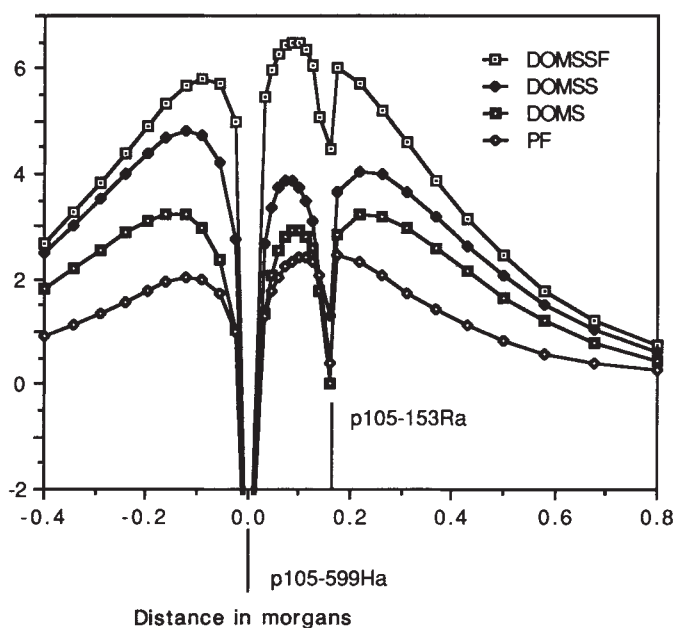


Fig. 2. Maximum likelihood estimates of the lod score were calculated from location scores from the LINKMAP programme of LINKAGE³⁷. Gene frequencies were as described in the Fig. 1 and Table 1 legends. A constant male:female recombination ratio of 1.5 was used with male distances as shown. The schizophrenia locus was moved from left to right, as shown, starting at an arbitrary distance of -0.4 Morgans from the p105-599Ha locus to a position of +0.8 Morgans to the right of p105-599Ha. p105-599Ha and p105-153Ra are at map positions 0.00 and 0.137 respectively. The maximum lod scores for schizophrenia and the two markers were found at 99% penetrance in the DOMS and DOMSS models and 86% in the DOMSSF analysis as shown. In the PF analysis complete penetrance was assumed. The highest lod score obtained was 6.49 with schizophrenia in the middle of p105-599Ha and p105-153Ra at approximately 0.08 Morgans from each marker under the DOMSSF model with 86% penetrance. In the PF analysis schizophrenia is placed at a genetic distance of approximately 0.07 Morgans to the right of p105-153Ra. Maximum likelihood estimates of penetrance for schizophrenia were calculated with ILINK. These were 86%, 76% and 73% for the DOMS, DOMSS and DOMSSF models respectively. These were incorporated into a further multipoint analysis (LINKMAP), with a correction for sporadic (unlinked to chromosome 5) cases of schizophrenia, by assuming a penetrance of 0.001 for the normal homozygote. Maximum lod scores were 3.22 (DOMS), 4.33 (DOMSS), 6.49 (DOMSSF) found at a map position approximately 0.08 Morgans from p105-599Ha in the direction of p105-153Ra. The data is not adequate to make a reliable determination of the gene order of schizophrenia in relation to the two marker loci.

We studied the inheritance of a number of RFLPs known to be on chromosome 5. Locus D5S76 is defined by *TaqI* polymorphisms detected by Southern hybridization by the probe p105-599Ha. Locus D5S39 is defined by *MspI* and *XbaI* polymorphisms detected by the probe p105-153Ra (refs 32, 33). These loci are linked together at the male genetic distance of about 13.7 centimorgans (cM)^{32,33}. The genotypes and pedigree structure of all the families are shown in Fig. 1.

We chose to use three different models for analysis because of the uncertainty of defining as cases those individuals with spectrum disorders as well as other psychiatric disorders. These models were: (1) DOMS, in which only individuals diagnosed with schizophrenia were scored as affected; (2) DOMSS, in which individuals with schizophrenia and schizophrenia spectrum disorders were regarded as cases; (3) DOMSSF, in which schizophrenia, schizophrenia spectrum disorders and all other fringe phenotypes were included. For each of the models

Table 1 Two-point lod scores under the DOMSSF model at specified recombination fractions for linkage between schizophrenia and the chromosome 5 markers

Family		Recombination fraction				
		0.00	0.10	0.20	0.30	0.40
F20	p105-599Ha	1.14	0.91	0.67	0.39	0.13
	p105-153Ra	0.69	0.40	0.13	-0.04	-0.07
F27	p105-599Ha	-2.95	0.57	0.82	0.66	0.31
	p105-153Ra	0.02	0.01	0.00	0.00	0.00
F35	p105-599Ha	-1.05	1.26	1.09	0.71	0.25
	p105-153Ra	0.20	0.24	0.20	0.11	0.03
F36	p105-599Ha	-0.17	-0.12	-0.07	-0.03	-0.01
	p105-153Ra	-0.36	0.60	0.58	0.39	0.14
F40	p105-599Ha	Not informative				
	p105-153Ra	0.54	0.42	0.28	0.15	0.04
F41	p105-599Ha	-0.04	2.09	1.71	1.10	0.40
	p105-153Ra	Not informative				
F74	p105-599Ha	-0.23	-0.07	-0.01	0.03	0.02
	p105-153Ra	0.82	0.62	0.42	0.23	0.07

Analysis using MLINK³⁷ under the DOMSSF model assuming a penetrance for schizophrenia of 86% in all families. The gene frequency for schizophrenia was 0.0085. A constant female: male recombination ratio of 1.5 was used. The two UK families give a lod score of between 1.5 and 2.4 according to the model used for analysis. The remainder of the lod score was contributed by Icelandic families. No statistical evidence for non-allelic heterogeneity of linkage was found (HOMOG³⁸, B-TEST³⁹).

described above we varied the degree of penetrance from 50% to 100%, in increments of 10%, and computed lod scores summed over all seven families for a putative schizophrenia susceptibility locus as the position of the locus was varied relative to the genetic map of the two marker loci. This is shown in Fig. 2 where the lods displayed are for penetrances that maximized the maximum lod score for each model. Additional analyses of the effects of penetrance are described in the Fig. 2 legend. In addition to analysing the data by varying penetrance we also used a 'penetrance free' (PF) model in which all 'unaffected' individuals were analysed as 'phenotype unknown' (Fig. 2). Although this discards a large fraction of the information it is robust to incomplete penetrance. Under this stringent approach we found the maximum multipoint lod score to be 2.45. A lod of 3.00 is good evidence for linkage for a disease with complete penetrance but when penetrance is used as a parameter the higher lod score of 3.85 is required (E. S. Lander, personal communication).

The data provide strong evidence for the segregation of a dominant schizophrenia susceptibility allele on chromosome 5 in some or all of the families. It is of particular interest that the highest lod score of 6.49 was found in the DOMSSF analysis at 86% penetrance. This suggests that the schizophrenia susceptibility allele may have alternative phenotypic manifestations and seems to represent a 'schizophrenia fringe' group of cases. However caution must be expressed in interpreting this result because only five out of the 10 potential fringe cases were informative for linkage analysis and because it is possible that this result may have occurred by chance. The range of phenotypic expression merits further attention because it may throw light on the genetic or environmental factors which modify or limit the expression of the schizophrenic susceptibility allele.

Caution is required in interpreting these results and applying them to other families. The locus on chromosome 5 may represent only one cause of schizophrenia. Additional genes on other chromosomes may exist and there may also be non-genetic forms. By selecting families with a large proportion of affected cases (primarily in Iceland), we may have enriched for a particular genetic subtype of schizophrenia. In other populations the penetrance of the chromosome 5 schizophrenia susceptibility allele may be lower than the value we observed in our families.

Despite the limitations on the validity of our findings for schizophrenia as a whole, the demonstration of a schizophrenia susceptibility locus on chromosome 5 provides the first concrete evidence for a genetic basis to schizophrenia. Moreover the presence of all the traditionally defined subtypes of schizophrenia in these families shows conclusively that clinical heterogeneity in schizophrenia can still have a common genetic basis. It should now be possible to explore the general applicability of our results to other families. Synthetic gene frequency maps established by principal components analysis show that the Icelandic population is genetically similar to the populations in northern England, north Germany and the south-east of Sweden³⁴. If this result can be confirmed and much more closely linked markers can be found then it may eventually be possible for psychiatrists to consider genetic counselling in families where chromosome 5 linkage can be reliably established. Lastly, attempts can be made to identify the 5q locus precisely by applying chromosome specific cloning techniques to isolate and characterize the mutation^{35,36}.

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Evidence against linkage of schizophrenia to markers on chromosome 5 in a northern Swedish pedigree

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Schizophrenia is a severe mental illness with a typically chronic course affecting nearly 1% of the human population. It is generally accepted that genetic factors have an important pathogenic role in a substantial portion of schizophrenia cases; however, despite decades of family studies, there is no agreed-upon mode of inheritance¹⁻⁶. The discovery of genetic aetiological factors and resolution of the inheritance pattern(s) will undoubtedly emerge from genetic linkage studies. With these objectives in mind, we undertook a linkage project, starting in 1985, in a previously well-documented kindred from north Sweden. Multipoint linkage analyses were used to screen the proximal long arm of chromosome 5 using restriction fragment length polymorphism (RFLP) markers at five loci and the distal long arm using RFLPs at two loci, one of which was the locus for the glucocorticoid receptor. We found strong evidence against linkage between schizophrenia and the seven loci. These results, together with the positive evidence⁷ for linkage of schizophrenia with markers in the proximal long arm of chromosome 5 lead us to conclude that the genetic factors underlying schizophrenia are heterogeneous.

The kindred used in our linkage studies is a geographical isolate located above the Arctic circle. It has low levels of alcohol abuse, no known drug abuse, and has been studied for several decades by Swedish psychiatrists. Descriptions of the kindred have been published elsewhere^{8,9}. Detailed epidemiological and genealogical data were gathered from local civil and parish records and through interviews with family members. Our work focused on seven branches of the kindred, all of whom can be traced back to three related families that moved into northern Sweden in the early seventeenth century. One of the branches is shown in Fig. 1.

Diagnostic information was obtained from medical records and examination of the patients and their relatives in their homes by one of us (L.W.). The majority of the patients were also examined by other psychiatrists for extended periods during hospitalization. Epidemiological data were available for 157 individuals in the seven branches. RFLP typing and diagnostic interviews were done for 81 subjects: 31 schizophrenics, all of whom met the narrow diagnostic criteria of both Feighner¹⁰ and DSM-III(ref. 11), and 50 unaffected relatives. Diagnostic status for both affected and unaffected was based on the following: a Swedish translation of a modified Schizophrenia and Affective Disorders Schedule—lifetime version (SADS-L) psychiatric interview, clinical follow-up from 1976 to now and hospital records. The younger patients exhibit particularly severe schizophrenia symptomatology with poor response to neuroleptic medication. Both positive and negative symptoms are prevalent. Average age of the 31 patients (15 females, 16 males) was 47

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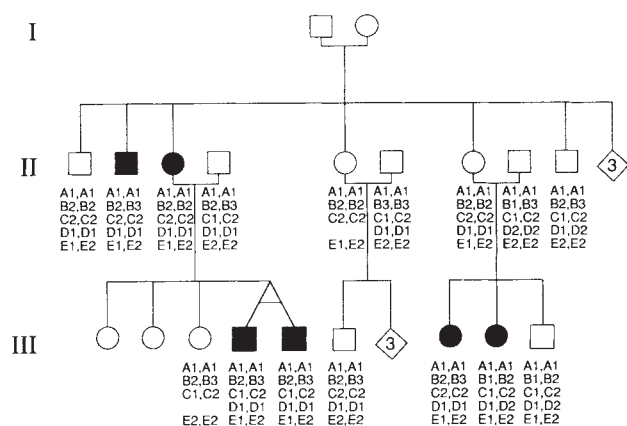


Fig. 1 Data on one of the seven branches of the north Sweden pedigree showing segregation of five RFLPs. Allele frequencies used in all analyses are: D5S21: A1 = 0.7, A2 = 0.3; D5S76: B1 = 0.32, B2 = 0.16, B3 = 0.52; D5S39: C1 = 0.33, C2 = 0.67; D5S78: D1 = 0.53, D2 = 0.47; and HEXB: E1 = 0.12, E2 = 0.88. Generation I are deceased. Twins shown were identical in all markers typed and for all analytical purposes were treated as one individual. Affected status for individuals in the first generation was considered unknown. B lymphocytes from blood samples were immortalized using a modification of the method of Anderson and Gusella²². Standard procedures were used for DNA extraction and RFLP typing²³. The probe p105-599Ha contains a repeat sequence and therefore was boiled for 10 min in the presence of sheared total human DNA before hybridization.

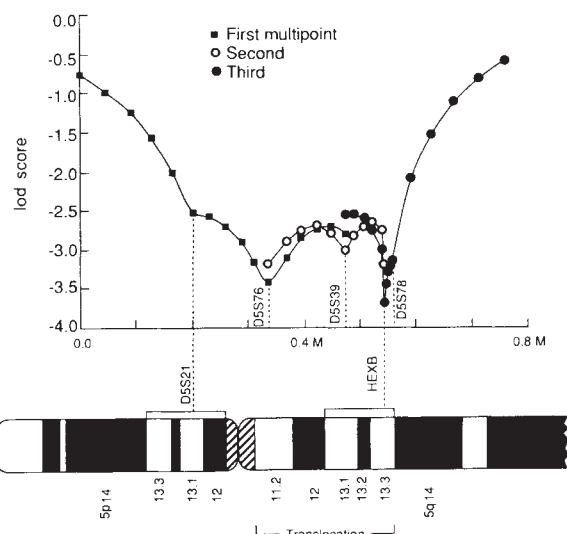


Fig. 2 Superimposed graphs of three 4-point analyses: the first moves the schizophrenia locus across a fixed map of D5S21, D5S76 and D5S39; the second across a fixed map of D5S76, D5S39 and HEXB; and the third across a fixed map of D5S39, HEXB and D5S78. D5S21 was arbitrarily set at 0.200 M. On the basis of genetic distances, D5S76 was set at 0.337 M, D5S39 at 0.474 M, HEXB at 0.544 M, and D5S78 at 0.561 M (all male distances; female/male ratio of recombination fraction across these markers is 1.5). Overlapping sections shown are those across the three inner markers. Attempts to do multipoint analyses with more than three markers at a time were interrupted because they were calculated to require more than 1,000 hours of central processing unit time in our VAX 8800.

years; average number of years in a mental hospital was 10. An additional 11 individuals were included in the analysis who had been interviewed by psychiatrists between 1946 and 1953 but were deceased when blood samples for RFLP typing were obtained. Six of these had schizophrenia and five were unaffected. In a further four of the living subjects the diagnosis remained uncertain after diagnostic interview and these were not included in the analyses. Two of these had significant depressive symptoms, but did not meet criteria for major depression; the other two had psychotic symptoms consistent with atypical psychosis. For three subjects sampled for DNA studies, cell lines could not be established. The remaining 58 individuals were deceased, defined as unknown diagnosis, and were used only to link the other living members within their branch. Unlike many reports of other pedigrees used in genetic studies of schizophrenia, the unaffected relatives in our study exhibited minimal affective disorder symptomatology, and none met criteria for schizotypal or schizoid personality disorder.

Chromosome 5 became an obvious place to focus our efforts because of two recent findings. Firstly, there has been a report¹² of an uncle-nephew pair of Asian origin, both of whom have schizophrenia and an abnormal karyotype consisting of trisomy for the 5q11.2-13.3 region, with the extra section of chromosome 5 translocated onto chromosome 1. Second, the gene for glucocorticoid hormone receptor (GRL) was reported to map to the same region of chromosome 5 (ref. 13). Non-suppression of glucocorticoid after dexamethasone administration occurs significantly more often in depressed than normal individuals¹⁴ and the potential for perturbations in the glucocorticoid system to cause psychotic symptoms is well known¹⁵. Thus, GRL could be considered a candidate gene for psychiatric disorders, including schizophrenia. In the course of our studies, however, we have obtained strong evidence that GRL is not in the translocation region, but instead maps to distal 5q (ref. 16).

We have studied eight markers mapped from the proximal short arm of chromosome 5 to the distal long arm. To determine the linkage relationships among the markers we typed them in a number of our large non-schizophrenic reference families as well as in the Swedish schizophrenia kindred. The resulting

map¹⁶ allows us to include seven of the eight in multipoint linkage analyses of schizophrenia. A map of chromosome 5 including five of the same loci has been reported¹⁷ and most of the raw data used to construct it were available to us through Centre d'Etude du Polymorphisme Humain (CEPH). Our independent analyses of the data on the CEPH families and results from our own reference families support this map and the relevant distances used in our analyses are the same (see Fig. 2). For a description of our reference families see ref. 18.

Schizophrenia was treated as an autosomal dominant trait with equal penetrances of 72% for homozygotes and heterozygotes. The disease allele was given a frequency of 0.02 to account for a morbid risk of 3% in the north Swedish population. Frequency of phenocopies was set at 0.1%. The penetrance value was obtained by maximizing the likelihood of the pedigree when there is no linkage information and the allele frequencies at the disease locus are fixed. It is worth mentioning that using this value for the penetrance does not maximize the magnitude of the lod scores reported below; penetrances above 72% in our pedigree increase the exclusion area. We used 56% as a lower bound to the penetrance because it gave a likelihood for the pedigree that was two natural logarithm support units below the maximum.

Table 1 shows the results of pairwise analyses between eight loci located on chromosome 5 and schizophrenia. Data shown are the sum of the lod scores obtained from independent analyses of seven branches of the kindred. Allelic segregation at several loci is depicted in one branch in Fig. 1. Locus D5S21 has been assigned to the centromere region of 5p; the other seven loci have been localized to 5q.

Figure 2 shows the results for three overlapping 4-point analyses of schizophrenia using the LINKMAP program^{19,20}. These multipoint analyses exclude schizophrenia (lod score < -2) from a region of about 40 cM in males (60 cM in females) extending from the proximal end of 5p, through the centromere,

Table 1 Pairwise analyses of schizophrenia and markers on 5q

Locus	Probe	Recombination fraction (males)							
		0.0	0.01	0.05	0.1	0.2	0.3	0.4	
D5S21	pJ0110HC	-1.72 (-0.80)	-1.58 (-0.75)	-1.19 (-0.60)	-0.86 (-0.45)	-0.42 (-0.23)	-0.16 (-0.09)	-0.03 (-0.02)	
D5S76	p105-599Ha	-2.73 (-1.98)	-2.52 (-1.84)	-1.82 (-1.35)	-1.22 (-0.91)	-0.52 (-0.38)	-0.18 (-0.12)	-0.03 (-0.01)	
D5S39	p105-153Ra	-1.58 (-1.05)	-1.45 (-0.94)	-1.02 (-0.65)	-0.63 (-0.40)	-0.21 (-0.13)	-0.05 (-0.02)	0.00 (0.00)	
D5S78	p105-798Rb	-1.17 (-0.77)	-1.05 (-0.67)	-0.70 (-0.39)	-0.44 (-0.21)	-0.18 (-0.06)	-0.06 (-0.02)	-0.01 (0.00)	
HEXB	pHexX	-1.45 (-0.99)	-1.10 (-0.72)	-0.56 (-0.31)	-0.29 (-0.12)	-0.07 (0.00)	0.00 (0.01)	0.00 (0.00)	
GRL	OB7	-2.61 (-1.81)	-2.36 (-1.66)	-1.69 (-1.20)	-1.16 (-0.82)	-0.54 (-0.38)	-0.20 (-0.14)	-0.04 (-0.02)	
D5S22	pJ0205ED	-4.69 (-3.33)	-4.32 (-2.99)	-3.09 (-2.05)	-2.05 (-1.31)	-0.84 (-0.49)	-0.27 (-0.13)	-0.05 (-0.01)	
DHFR	pHFR1.8	-1.91 (-1.57)	-1.54 (-1.30)	-0.91 (-0.77)	-0.53 (-0.43)	-0.14 (-0.10)	0.00 (0.00)	0.00 (0.00)	

Data shown are lod scores at penetrance of 72%; lod scores at lower bound penetrance of 56% are in parentheses. Allele frequencies and descriptions of all systems have been published and are available through the Howard Hughes Medical Institute Human Gene Mapping Library at 25 Science Park, New Haven 06510, USA. The analyses assumed a female/male theta ratio of 1.5 for all markers except GRL and D5S22 in 5q where the ratio is 1.0.

past the two markers used by Sherrington and colleagues⁷, to about 4 cM distal to D5S78. The length of this exclusion area is about 1.5% of the entire human genome assuming a 3,300 cM total map. It is worth noting the advantage of the multipoint analysis carried out with the benefit of the genetic map: the exclusion obtained with these same five loci in pairwise analyses (Table 1) is 6 cM in males and 9 cM in females, only about 15% of the exclusion area provided by a multipoint strategy using the same raw data. The hexosaminidase B (HEXB) locus maps to 5q13 and has been shown²¹ to be within the 5q11.2-13.3 trisomic segment¹². Therefore, the exclusion region between D5S21 and D5S78 shown in Fig. 2 probably encompasses the majority, if not all, of 5q11.2-13.3, essentially eliminating the possibility of linkage of the trisomic region to schizophrenia in our kindred.

In contrast to the previous report using physical mapping techniques¹³, we have used linkage studies to map the GRL locus to distal 5q, near D5S22 (ref. 16). A multipoint analysis moving the schizophrenia locus across the D5S22-GRL pair in distal 5q generates lod scores < -2 and excludes a region of 32 cM (in both males and females) adding to the pairwise evidence excluding linkage of the candidate gene GRL to schizophrenia in the north Swedish kindred (Fig. 3).

As is obvious from the results of maximum likelihood penetrance compared to lower bound in Table 1, the exclusion is dependent on the penetrance (among other variables). At present there is no certain strategy for obtaining an unbiased estimate of penetrance for a psychiatric disorder. Therefore, in addition to calculations at the lower bound of 56% penetrance,

we examined even lower penetrances. Our analyses do not yield statistically significant (lod score < -2) exclusion of linkage at lower values of the penetrance; they do, however, continue to give negative lod scores across both regions studied. Until there are valid methods for establishing confidence limits to the inheritance parameters required in a linkage analysis, results of linkage studies of psychiatric disorders need to be interpreted with great caution.

Not only did we fail to find any positive evidence linking schizophrenia to 5q markers in this kindred, but also we found strong exclusionary evidence in large domains of the parameter space. Therefore we conclude, in the northern Swedish kindred, a single major locus underlying schizophrenia does not lie in the region between D5S21 and D5S78 or in their immediate vicinity, nor is it linked to other 5q loci DHFR, D5S22, or GRL. Because HEXB has been reported to map to the distal end of the 5q11.2-13.3 region, our lod scores of < -2 from the proximal short arm through the HEXB locus indicate absence of linkage to the trisomic segment. In the presence of positive linkage findings by other groups using some of the same markers⁷, our data provide strong evidence for heterogeneity in the genetic aetiology of schizophrenia. Thus, the pathogenesis of schizophrenia in the families of Sherrington and colleagues and in the north Swedish kindred probably involves mutations at different chromosomal locations. At present it seems that there are different loci, possibly leading to different neurophysiologic abnormalities, resulting in a 'final common pathway' phenotype of schizophrenia.

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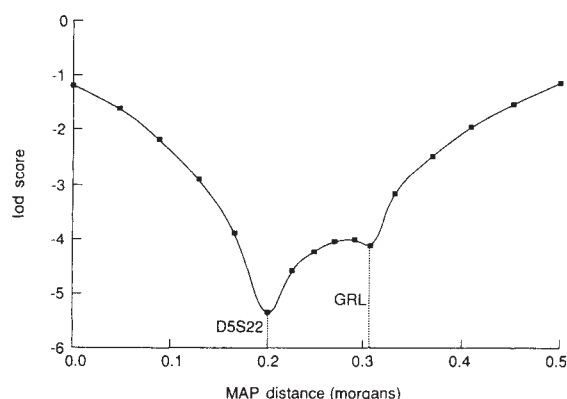


Fig. 3 Multipoint analyses of schizophrenia across a fixed map of D5S22 (arbitrarily set at 0.200 M) and GRL (set at 0.307) in distal 5q. We used a female/male ratio of the recombination fraction equal to 1, based on recombination data from our reference families¹⁶.

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GABA-containing neurons in the septum control inhibitory interneurons in the hippocampus

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The hippocampus, in particular the neocortex–hippocampus–neocortex circuit, is widely believed to be crucial in memory. Information flow in this circuit is strongly influenced by relatively sparse afferents derived from subcortical centres, such as the septum, involved in arousal, emotions and autonomic control. A powerful mechanism, by which numerically small inputs can produce profound effects, is feed-forward inhibition¹, that is, the activation of local inhibitory interneurons, which, in turn, control the activity of large populations of principal cells in the hippocampus. An example is the cholinergic input to the hippocampus from the septum, which is likely to be involved in feed-forward operations^{2–8}. Here, we demonstrate the existence of a circuit underlying another powerful mechanism of subcortical control of hippocampal information processing. We show that GABA-containing afferents originating in the septum innervate most of the GABA-containing interneurons in the hippocampus, making many synaptic contacts with each of them. Activation of the GABA-containing neurons in the septum is likely to lead to disinhibition of the principal neurons in the hippocampal formation and so this pathway is probably crucial in the induction of hippocampal electrical activity patterns, and may be involved in NMDA (*N*-methyl-D-aspartate) receptor-mediated functions, such as memory⁹, in a permissive manner.

Electrophysiological studies have suggested that there is a non-cholinergic component in the septohippocampal pathway, which facilitates responses evoked by stimulation of the major excitatory pathways in the hippocampus^{10,11}. Recently, non-cholinergic¹², presumably GABA-containing¹³, neurons have been shown to participate in the septohippocampal projection, but their relative abundance and sites of termination in the hippocampus are not known. We attempted to determine whether the non-cholinergic component of the septohippocampal pathway is in fact GABA-containing, and if so, whether the site and pattern of its termination provides an anatomical basis for the observed disinhibition.

A characteristic type of septal axon, visualized by anterograde transport of *Phaseolus vulgaris* leucoagglutinin (PHAL), was shown to establish numerous basket-like formations around interneurons (Fig. 1a and Fig. 3; see also ref. 14) in all regions of the hippocampal formation. Alternate ultra-thin sections of a series cut from this material were immunostained for GABA using a post-embedding immunogold procedure (see Fig. 1 legend). Boutons of this type of afferent, as well as most of their

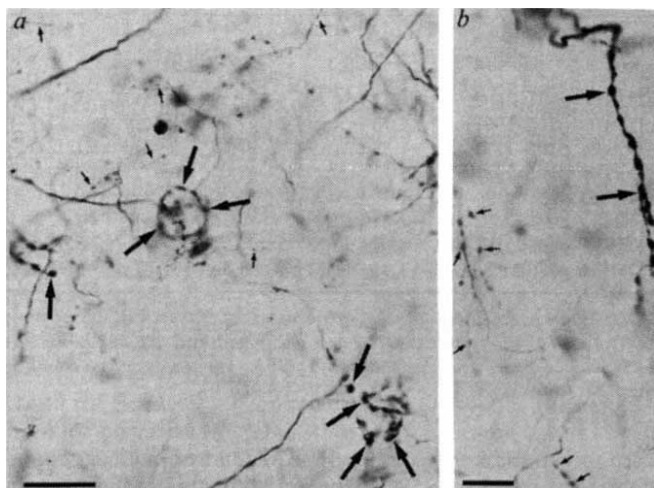


Fig. 1 Light micrographs from the CA3 region (stratum radiatum) of the rat hippocampus. Two types of septohippocampal axons, visualized by immunostaining for anterogradely transported PHAL, can be seen in these 60-μm vibratome sections. Type 1 axons stained stronger for PHAL, have large boutons (large arrows), and frequently surrounded cell bodies (two of them are seen in a). Type 2 axons were fainter, had smaller boutons (small arrows), and were scattered mainly in the neuropil. Scale bars: a, 20 μm; b, 10 μm.

Methods. Septohippocampal axons were visualized by anterograde transport of PHAL (2.5%, Vector) iontophoretically into the medial part of the medial septum and the vertical limb of the diagonal band, areas which have been reported to contain the highest proportions of non-cholinergic septohippocampal neurons¹². Twelve adult female rats (Sprague–Dawley, 200–250 g) were used. Six to eight days later the animals were perfused through the heart first with Tyrode's solution (1–2 min), then with a fixative containing 1% glutaraldehyde, 2% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer (pH 7.4) for 30 min. Blocks of the injection site and the ipsilateral hippocampus were dissected, immersed in 10 and 20% sucrose, freeze-thawed in liquid nitrogen and sectioned at 60 μm on a vibratome. Following extensive washes in the same buffer and a treatment with 1% sodium borohydride (30 min), the sections were immunostained using the following mixtures of antibodies. Primary antibodies (for 2–3 days): biotinylated anti-PHAL (1:200, Vector), plus rabbit anti-calbindin²⁸ (R 202, 1:1,000), or biotinylated anti-PHAL (1:200, Vector), plus rabbit anti-parvalbumin²⁸ (R 301, 1:400). Second layer (for 4–6 h): avidin biotinylated-horseradish peroxidase complex (ABC, Vector, 1:100), plus goat anti-rabbit IgG (ICN, 1:50). The first immunoperoxidase reaction was developed using 3,3'-diaminobenzidine (DAB) as a substrate, intensified with ammonium nickel sulphate (deep blue to black reaction product; for a detailed description of the double staining procedure see ref. 14). Third layer (for about 12 h): rabbit peroxidase anti-peroxidase complex (DAKOPATTS, 1:100). The second immunoperoxidase reaction was developed with DAB alone (brown reaction product). Tris (0.05 M)-buffered saline (pH 7.4) containing 1% normal goat serum and 0.5% Triton X-100 was used for all the washes and antibody dilutions. Alternate sections processed for only PHAL without Triton were treated with 1% osmium tetroxide for 1 h, then dehydrated, embedded in Durcupan (ACM, Fluka) and mounted on slides. Selected areas were re-embedded and sectioned for subsequent electron microscopy and post-embedding immunogold staining for GABA. Semi-thin sections (0.5 μm thick) cut from target somata, were also immunostained for GABA. The immunogold staining procedures followed those previously described²⁹, using a well characterized antiserum against GABA³⁰. The controls included incubations in which the primary antiserum was replaced by the same anti-GABA antiserum absorbed to GABA (1:1,000), or by normal rabbit serum (1:500). Accumulation of gold particles on PHAL-immunoreactive terminals or any other profiles was not seen in these sections.

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postsynaptic targets—either cell bodies or dendrites—were immunoreactive for GABA (Fig. 2).

PHAL-labelled axons studded with boutons were found in all layers and regions of the hippocampal formation. There were two distinct types of axons¹⁵. One type, which we showed was GABA-containing, had large *en passant* boutons (type 1); the other type (type 2) was characterized by smaller varicosities with occasional drumstick-like terminals, as shown in Fig. 1. The most striking feature of the type 1 (GABA-containing) arbors was that they frequently formed multiple basket-like contacts around cell bodies and proximal dendrites (Fig. 1a) in all layers, but predominantly in strata oriens and radiatum of CA3, and in the hilus and granule cell layer of the dentate gyrus. Type 2 axons, which probably represent the cholinergic component of the septohippocampal pathway, were seen in the proximal dendritic regions of pyramidal and granule cells, and less frequently in stratum lacunosum moleculare of CA1 and CA3. They also contacted cell bodies of interneurons, although more rarely than type 1 axons. Terminals of type 2 axons were found to be negative for GABA, as well as for most of their targets, which were mostly dendritic shafts.

To obtain a more global view of the relationship between the PHAL-labelled septal axons and the hippocampal interneurons, alternate vibratome sections (60 μ m thick) were stained for both PHAL and parvalbumin, or for PHAL and calbindin, using different chromogens for the two immunoperoxidase reactions¹⁴. Both parvalbumin and calbindin—vitamin D dependent, relative molecular mass (M_r) of 28,000 (28K)—are calcium-binding proteins. Parvalbumin is known to be selectively present in GABA-containing interneurons in the hippocampal formation and neocortex^{16,17}. Calbindin is localized in a population of interneurons that is probably different from the population containing parvalbumin¹⁸.

Positive antibody reactions for these proteins were taken as evidence that the cells were interneurons and not pyramidal cells. Using pre-embedding immunostaining for these proteins, most of the dendritic tree of interneurons can be visualized. In the double-stained sections, we found that most of the interneurons (immunoreactive for parvalbumin or calbindin), received multiple contacts from type 1 (GABA-containing) septohippocampal afferents (Fig. 3). The PHAL-labelled boutons, from two to as many as 46 per cell, were distributed mainly in the somatic region, but distal dendrites were often also ensheathed. In eight sections (60 μ m thick) 384 parvalbumin-immunoreactive cells were counted in the CA3 region and 268 in the dentate gyrus; 71% and 62% of these, respectively, were innervated by PHAL-labelled septal axons. In eight neighbouring sections 464 calbindin-immunoreactive cells were counted in the CA3 region, and 69% of these were innervated by PHAL-labelled septal axons. These should be considered to be minimum numbers, because in any one area only a proportion of the septohippocampal fibres were labelled with PHAL, and parts of the dendritic tree of the target interneurons were in neighbouring sections, where they may also receive septal input. In this respect it is interesting that, in some sections of the CA3 region, as many as 84% of the calbindin- or parvalbumin-immunoreactive cells were innervated by PHAL-labelled varicose fibres. Interneurons in the CA1 region and in stratum moleculare of the dentate gyrus were less frequently innervated by PHAL-labelled type 1 axons, and only a very few such contacts were seen in stratum lacunosum moleculare of CA1 and CA3 in any of the double-stained sections. Calbindin is also present in granule cells of the dentate gyrus and in a large number of pyramidal cells in the CA1 region¹⁸. However, such principal cells were never found to receive multiple synaptic input from GABA-containing septohippocampal axons.

These results have important functional implications concerning the mechanisms of septohippocampal actions. Stimulation of the GABA-containing septohippocampal pathway described in this study would probably reduce local hippocampal inhibi-

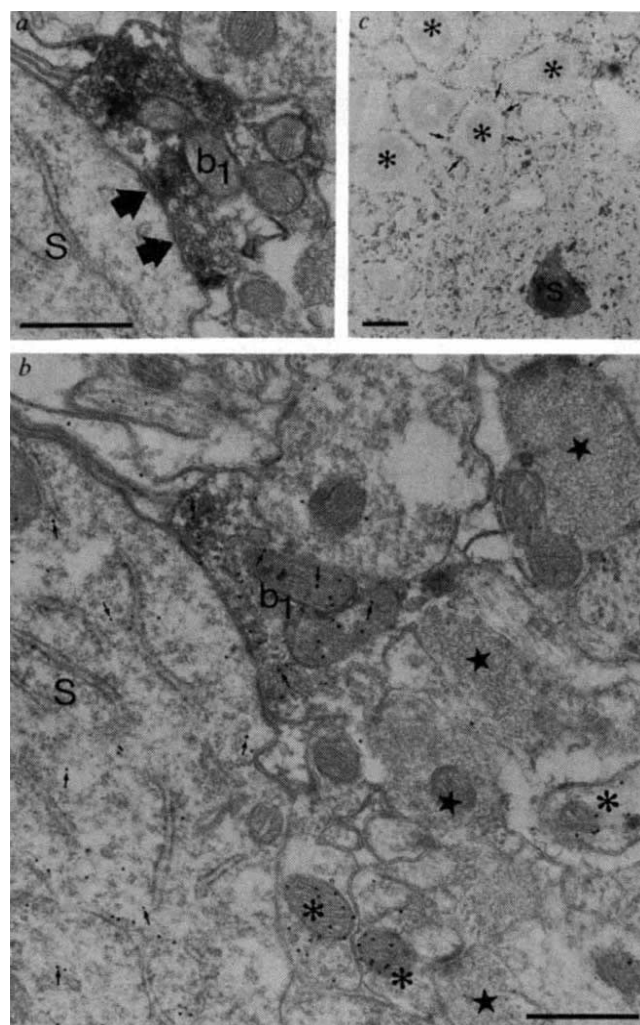


Fig. 2 *a*, Electron micrograph of an ultrathin section cut from a PHAL-immunoreactive type 1 bouton (b_1) in the CA3 region. It is in symmetrical synaptic contact (large arrows) with a cell body (S). *b*, Electron micrograph of the same PHAL-labelled bouton from a different section of the series, which was stained by the immunogold procedure for GABA. Both the PHAL-labelled terminal (b_1), and the postsynaptic soma (S) are immunoreactive for GABA, as shown by the accumulation of colloidal gold particles (a few of them are indicated by small arrows). Other GABA-immunoreactive profiles are also labelled (asterisks). Terminals, which establish asymmetrical synapses and contain large round vesicles (stars), are always negative for GABA. *c*, A semi-thin section was cut from the remaining part of the same cell body (S) shown to be postsynaptic to the GABA-containing PHAL-labelled terminal in *a* and *b*, and was immunostained for GABA. It is strongly positive, whereas the pyramidal cell bodies (some labelled by asterisks) are negative, and are surrounded by GABA immunoreactive boutons (small arrows). Scale bars: *a*, *b*, 0.5 μ m; *c*, 10 μ m.

tion of both feed-back and feed-forward type to a minimal level^{3,10,11}. What could be the effect of this powerful disinhibitory pathway during spontaneous physiological activity? In conjunction with cholinergic mechanisms³ a GABA-containing disinhibitory mechanism may be crucial for the rhythmic synchronization of hippocampal neurons during theta activity. Recent studies have shown that long-term potentiation (LTP) can be preferentially induced when the cells are maximally excited^{19,20}. For granule cells, this occurs during the positive phase of the local theta waves, whereas for pyramidal cells it is during the

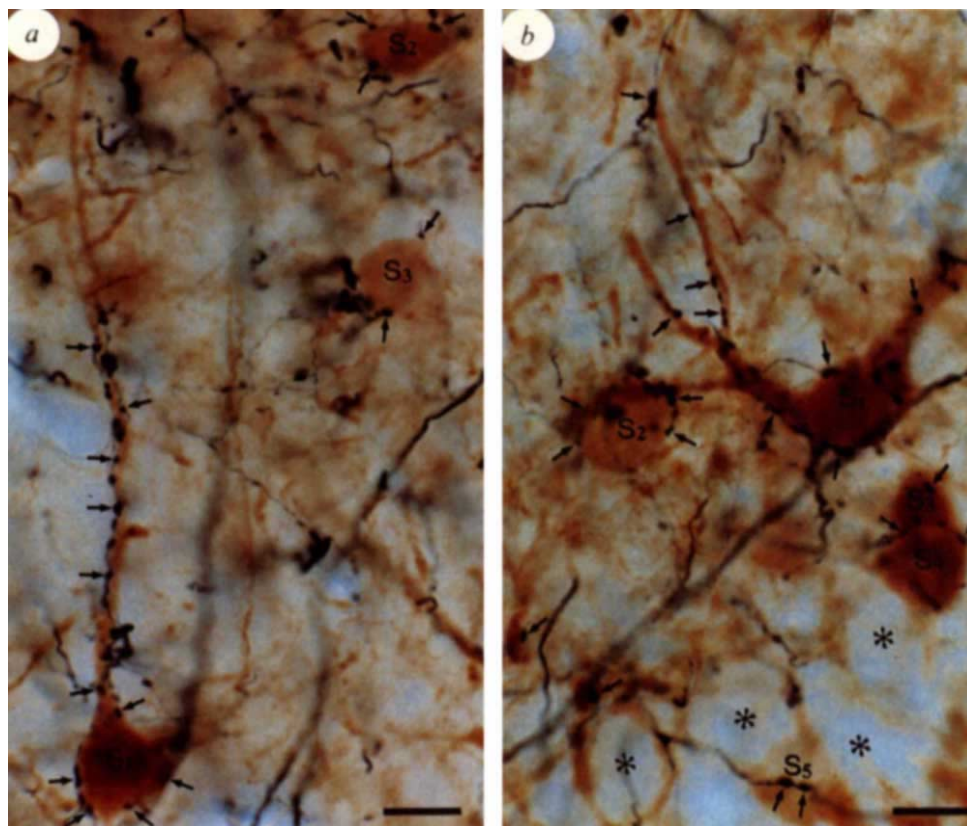


Fig. 3 Light micrographs of vibratome sections from the CA3 region of the rat hippocampus immunostained for *a*, calbindin (calbindin D-28K) and PHAL, or *b* parvalbumin and PHAL. The dark blue to black fibres with large varicosities (arrows) are PHAL-immunoreactive, type 1, septohippocampal afferents. They surround the somata (S_{1-5}) and dendrites of calbindin (in *a*) and parvalbumin (in *b*)—immunoreactive interneurons, which are stained brown. Some of the immuno-negative pyramidal cells are also indicated (asterisks). Scale bars: 10 μ m.

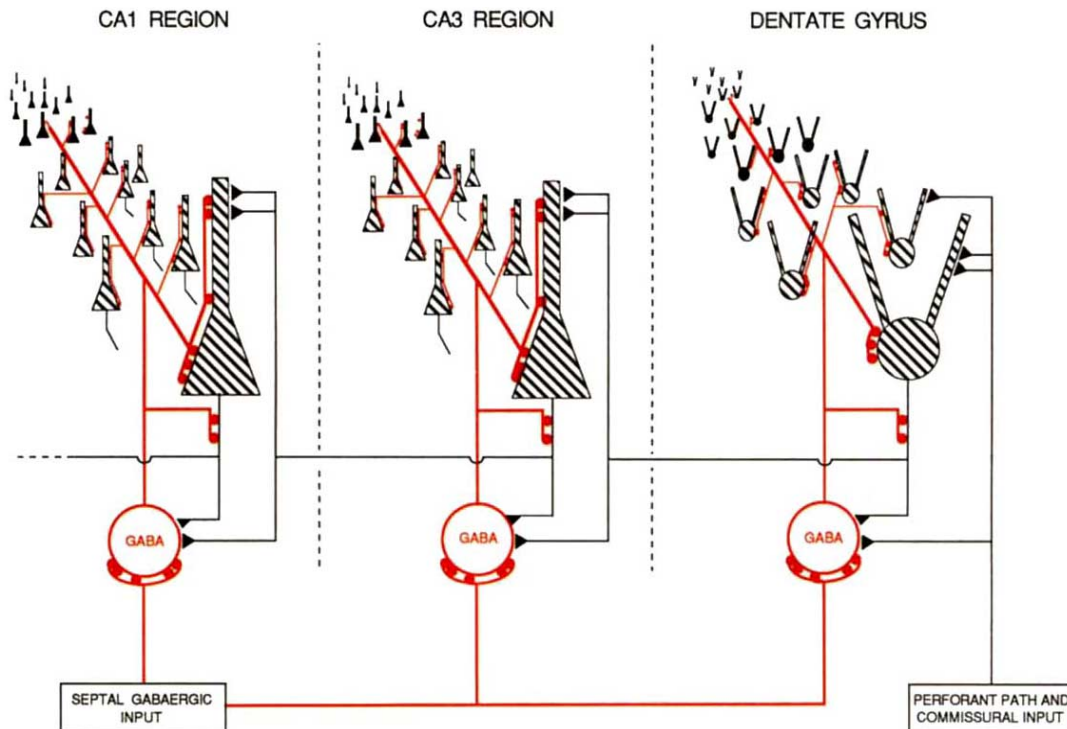


Fig. 4 Summary diagram of the circuit described in this study. The inhibitory GABA-containing neurons and axons are drawn in red and the excitatory neurons and connections in black. The relatively sparse GABA-containing septohippocampal afferents, which are likely to enter the hippocampus through the fimbria-fornix, can have widespread effects in the hippocampus by innervating GABA-containing inhibitory interneurons which in turn control the activity of large populations of principal cells. This powerful, presumably disinhibitory, action takes place at each of the three stages of hippocampal information processing, in the dentate gyrus, CA3 region and CA1 region. The types of GABA-containing interneurons innervated by septal afferents remain to be identified. They probably include several distinct types (basket cells, axoaxonic or chandelier cells and so on) contacting various regions of the output cells, and are represented by a general type (red) in the diagram.

negative phase of local theta or even more during sharp waves⁸. A septal depression of local GABA-mediated transmission during these periods would certainly increase excitability^{3,10,11}, providing optimal conditions for the afferent activation to depolarize the cells to a level where NMDA-receptor-mediated responses can be evoked²¹⁻²³. In the cerebral cortex of adult animals LTP cannot be reliably induced under normal conditions²⁴. However, if the powerful feed-forward inhibition is removed from local cortical circuits by bicuculline, LTP can readily be induced²⁵.

A close relationship has been demonstrated between the magnitude of GABA containing hyperpolarization and NMDA-receptor-mediated evoked responses²², as well as NMDA-mediated epileptiform burst firing^{26,27}. The GABA-containing sep-

tohippocampal pathway can, therefore, through disinhibition, have a powerful effect on NMDA-receptor-mediated functions, and any disturbance in the activity of this pathway is expected to lead to pathological excitability in the hippocampal formation.

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Temporal hyperacuity in single neurons of electric fish

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Behavioural studies have revealed that animals can resolve temporal disparities in the microsecond range¹⁻⁴. This resolution is far superior to that of individual receptors, and it must therefore be achieved through central neuronal mechanisms. It is unclear, however, whether such sensitivity ever emerges at the level of single neurons, or whether it is apparent only at the behavioural level through the collective action of many less-sensitive neurons. We have found that single neurons in the pre-pacemaker nucleus of a weakly electric fish are sensitive to temporal disparities as small as 1 μ s, the highest temporal sensitivity ever observed at the single-neuron level. The remarkable temporal resolution of these pre-pacemaker neurons results from a high degree of spatial convergence of afferent inputs. These neurons represent the final elements of a sensory hierarchy and directly control the jamming avoidance response by which these fish regulate the frequency of their electric organ discharges⁵⁻⁷.

The electric fish *Eigenmannia* emits quasi-sinusoidal electric organ discharges (EODs) of several hundred Hz from an organ located in the tail; it senses the EODs with electroreceptors distributed over the body surface. The ability of the fish to detect objects as perturbations of its EOD field can be severely impaired by a neighbour's EOD of similar frequency. Accordingly, fish with similar EOD frequencies shift them apart to minimize mutual jamming. A fish will lower its own frequency if exposed to a signal of slightly higher frequency and will raise its frequency if the signal is of a slightly lower frequency^{8,9}.

This jamming avoidance response (JAR) can be elicited in curarized *Eigenmannia*^{8,10}. Although most of the EOD is silenced by curarization, the neuronal pacemaker in the medulla, which normally triggers each EOD cycle by a single command pulse, continues to fire at its normal frequency. If the silenced

EOD is replaced by a constant sine wave, S_1 , of similar frequency, the pacemaker will display a normal JAR in response to a second sine wave, S_2 , mimicking the EOD of a neighbour, by raising and lowering its frequency in accordance with the sign of the frequency difference, D_f , between S_1 and S_2 ($D_f = f_{S_2} - f_{S_1}$).

Behavioural experiments have shown that, to determine the sign of D_f and thus to perform a correct JAR, *Eigenmannia* must detect modulations in both phase differences and amplitude that result from the interference of the two signals, S_1 and S_2 . A phase difference is detected as a difference in the timing of zero-crossings of signals received at different regions of the body surface^{10,11} (Fig. 1). As the relative amplitude of the interfering signal S_2 approaches zero, the depth of these modulations approaches zero proportionately, and the animal eventually fails to determine the sign of D_f . *Eigenmannia* can determine the sign of D_f even when the modulations in phase difference, or temporal disparity, are smaller than 1 μ s (ref. 1).

To address the question of whether this sensitivity to small temporal disparities exists at the single-neuron level, we made recordings from neurons in the pre-pacemaker nucleus which is the final element in the sensory hierarchy that processes information about the sign of D_f (refs 5, 12). For a precise experimental control of temporal disparity, curarized fish were placed into a multi-compartment chamber (Fig. 2 insert) which allowed separate stimulus regimes to be applied to different regions of the body surface¹¹. S_1 and S_2 were electronically mixed and applied to the head compartment so that all receptors in this part of the body surface experienced identical phase modulations. An uncontaminated S_1 , which lacked phase modulation and acted as a phase reference, was offered to all trunk compartments. In this way, we created precise temporal disparities between the head and trunk compartments. By decreasing the relative intensity of S_2 , the temporal disparity was reduced to threshold levels. The sign of D_f was switched every two seconds, and the responses of 'sign-selective' neurons¹² in the pre-pacemaker nucleus were monitored. These neurons, which are excited by negative D_f s and inhibited by positive D_f s, modulated their rate of firing with each change of the sign of D_f , even when temporal disparities were as small as 1 μ s. The

Fig. 1 Stimulus variables controlling the jamming avoidance response (JAR). *a*, Assume that area B of the animal's body surface experiences only the animal's own electric organ discharge (EOD), whereas area A is exposed to a mixture of the animal's own EOD and that of a neighbour of slightly different frequency. Both EODs are simplified as sine waves. A recording at the electroreceptors in area B will then reveal the animal's own uncontaminated sine wave (upper right), but a recording in A will show a mixture of two sine waves (underneath), a sinusoidal signal whose amplitude, $|S|_A$ and phase in reference to the signal in B (the difference in the timing of corresponding zero crossings), $H_A - H_B$, are modulated at the frequency difference between the two sine waves. As pairs of these values are sampled at successive cycles, t_1 , t_2 and so on, and then plotted in a two-dimensional state plane *b*, a circular graph is obtained which rotates in the clockwise sense if the neighbour's EOD is of lower frequency than the animal's own EOD. An anticlockwise sense is obtained if the neighbour's EOD is of higher frequency. The sense of rotation of this graph thus reveals the sign of the frequency difference, D_f , between the interfering EODs and determines in which direction the animal must shift its own EOD frequency to move away from that of its neighbour. The areas A and B were chosen arbitrarily as an example and could be anywhere on the body surface. If the animal's own signal amplitude is normalized to one and if the phase is measured in radians, the radius of the circle is equal to the ratio, r , between the local amplitude of the interfering EOD and that of the animal's own EOD. For an EOD cycle of period P and a given amplitude ratio r , the maximal difference in the timing of zero-crossings, which corresponds to the radius of the circle, is $P \cdot r / 2\pi$ (refs 11 and 14). The circle thus contracts to a point as the neighbour's signal vanishes. *c*, *Eigenmannia* is able to 'read' the sense of rotation of such circular graphs after encoding the amplitude by the rate of firing, p_A , of local P-type receptors and by computing the phase difference from the difference, $T_A - T_B$, in the timing of spikes of T-type receptors in A and B, which are phase-locked to the moment of the zero-crossing of the local signals. A joint evaluation of amplitude and phase modulations by higher-order, sign-selective neurons in the midbrain and the diencephalon yields the sense of rotation and ultimately controls appropriate shifts of the animal's EOD frequency. (see Fig. 4 for neuronal flow of information).

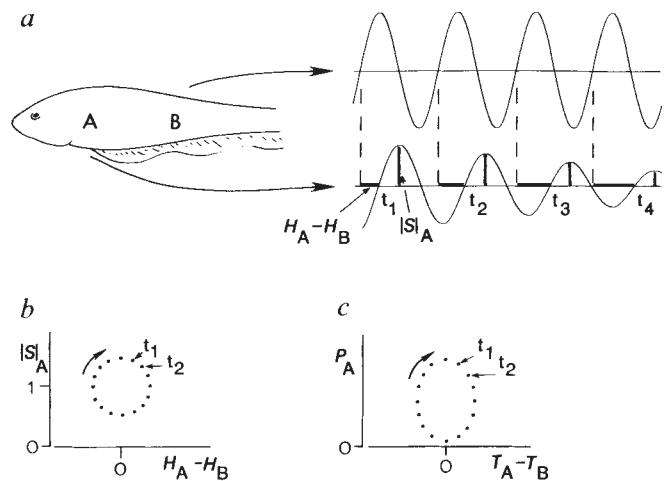
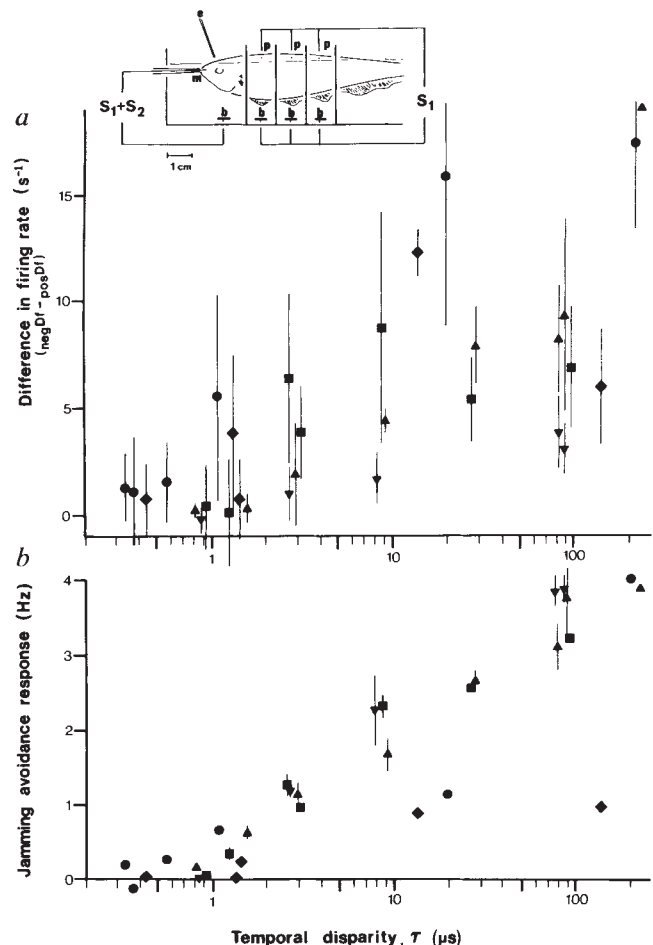


Fig. 2 *a*, Neurons in the pre-pacemaker nucleus are sensitive to temporal disparities of $1 \mu\text{s}$. Means and standard deviations of differences in firing rate (rate during negative D_f minus rate during positive D_f) are plotted against peak temporal disparity (τ) in the modulation of phase difference, that is the timing difference between zero-crossings of stimuli applied at the head and trunk region. m, Mouth electrode; b, bottom electrodes; p, electrodes in dorsal musculature; e, recording electrode. Different symbols represent different neurons. With reference to its spontaneous rate of firing, neuron (●) raised and lowered its rate by 140% and 49% for negative and positive D_f , respectively, at a temporal disparity of $1.1 \mu\text{s}$. *b*, Magnitude of the JAR: mean frequency shift of the pacemaker during 2-s periods of negative D_f alternating with 2-s periods of positive D_f . The behavioural data were taken concurrently with the neuronal measurements in *a* and are marked with the same symbols.

Methods. Curarized fish ($n = 4$) were placed in a multi-compartment chamber (insert). A sinusoidal EOD replacement signal, S_1 , and a sinusoidal jamming signal, S_2 , with $|D_f|$ of 4 Hz, were electronically added and applied between an electrode in the mouth (m) and an electrode at the bottom (b) in the head compartment. Thus, receptors on the head experienced modulations in phase and amplitude of 4 Hz. Receptors in three trunk compartments were stimulated with an identical S_1 applied between pin electrodes inserted in the dorsal musculature (p) and the bottom electrodes (b), thus experiencing no phase or amplitude modulation. The maximal temporal disparity between the head compartment and the trunk compartments was: $\tau = P/2\pi \cdot |S_2|/|S_1|$, where P is the S_1 period (2.4 to 3.3 ms), $|S_2|/|S_1|$ is the relative amplitude between S_2 and S_1 (refs 11 and 14), which was varied from 0.001 to 0.3. EODs, greatly attenuated by curarization, could still be monitored by a suction electrode fitted over the tail to record the jamming avoidance response (JAR). Two to 11 pairs of 2-s-long stimulus periods with negative and positive D_f s, were presented consecutively for each data point.



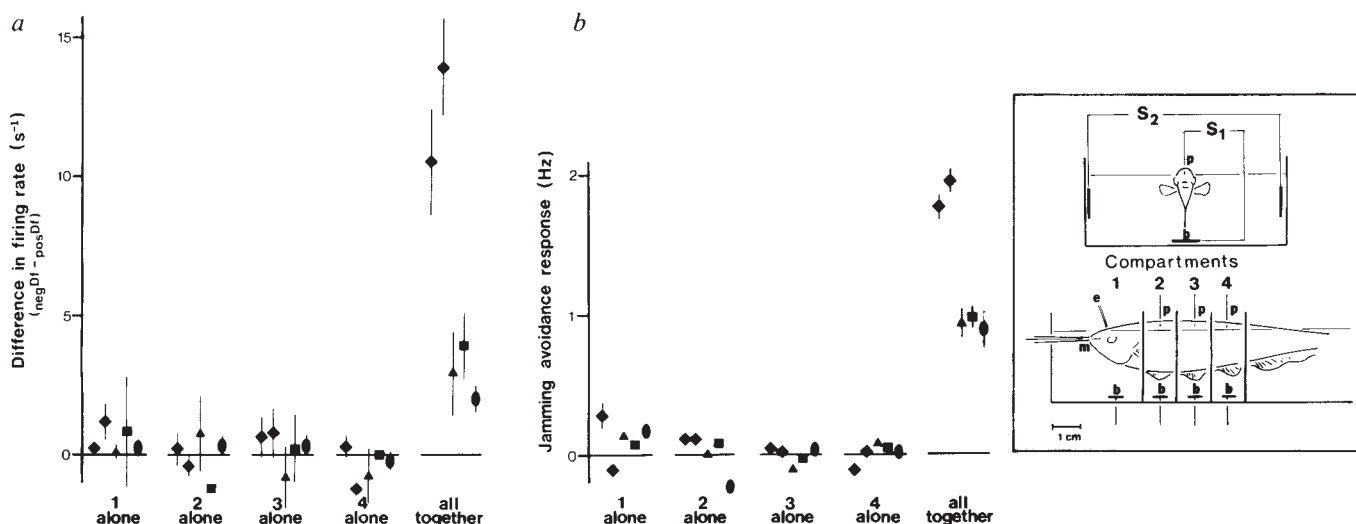


Fig. 3 Simultaneous recordings of pre-pacemaker neurons (*a*) and the JAR (*b*) during stimulation of different regions of the body surface (1, 2, 3, 4). Regions were stimulated either individually or jointly. *a*, Means and standard deviations of differences in firing rate (rate during negative D_t minus rate during positive D_t). *b*, Magnitude of JAR as described in Fig. 2. Different symbols represent different single-unit recordings and concurrent measurements of JAR.

Methods. The same neurons as presented in Fig. 2 were tested in the same multi-compartment chamber, but with a different stimulus configuration. In each compartment, S_1 was applied between mouth or pin electrodes and electrodes at the bottom, and S_2 was applied through two electrodes straddling the fish in each compartment (insert). Because of the different geometry of S_1 and S_2 , different parts of the body surface experienced different ratios of $|S_2|/|S_1|$. Therefore, differential phase modulations, or temporal disparities, existed within each compartment. The maximal temporal disparities were $\sim 6 \mu\text{s}$ ($\blacktriangle$, $\bullet$, $\blacksquare$) or $28 \mu\text{s}$ ($\blacklozenge$). Identical S_1 and S_2 were delivered either to single compartments or jointly to all four compartments by means of external switches. Two to six pairs of stimulus periods with negative and positive D_t s, each period 2 s long, were presented consecutively for each data point.

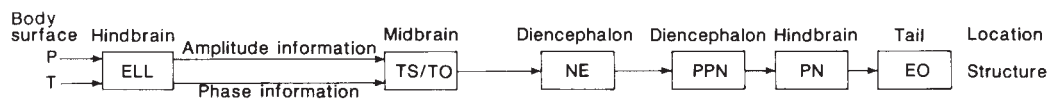


Fig. 4 The pre-pacemaker nucleus (PPN) represents the top of a neuronal hierarchy controlling the frequency of EODs. P- and T-type receptors on the body surface code amplitude and phase of electric signals, respectively. The electrosensory lateral line lobe (ELL) in the hindbrain processes amplitude and phase information independently and relays phase information to lamina 6 of the torus semicircularis (TS) of the midbrain. Neurons in lamina 6 compute differential phase information which converges with amplitude information in deeper layers of the TS and the tectum opticum (TO). This convergence yields two classes of 'sign-selective' cells, excited by $D_t < 0$ and $D_t > 0$, respectively. The nucleus electrosensorius (NE) of the diencephalon contains two classes of higher order sign-selective cells, excited by $D_t < 0$ and $D_t > 0$, respectively. Neurons in the pre-pacemaker nucleus (PPN) are excited by $D_t < 0$ and inhibited by $D_t > 0$. Their excitation raises the frequency of the pacemaker nucleus (PN) in the hindbrain which triggers each discharge cycle of the electric organ (EO) by a single command pulse.

simultaneously recorded JAR had a similar threshold (Fig. 2).

A previous study⁷ had shown that the JAR requires stimulation of a large area of body surface when temporal disparities are small. To assess whether this spatial integration is observed at the level of single pre-pacemaker neurons, the stimulus regime used in Fig. 2 was modified (insert in Fig. 3). In each compartment, S_1 and S_2 were applied through different pairs of electrodes, so that the current vectors of S_1 and S_2 formed different angles in different regions of the body surface. As a result, the relative intensities of S_1 and S_2 differed over the body surface, creating temporal disparities within each compartment^{9,10}. In accordance with the JAR, the pre-pacemaker neurons determined the sign of D_t with the stimulation of any one compartment when temporal disparities were large. As temporal disparities were reduced to below a certain level, pre-pacemaker neurons could no longer determine the sign of D_t , and the JAR disappeared. When the identical signals were applied jointly to all four compartments, however, pre-pacemaker neurons could again determine the sign of D_t , and the JAR reappeared (Fig. 3).

Temporal resolution of the order of $1 \mu\text{s}$ seems very short compared with the much longer time constants of electrical events in neurons. In the light of the relative slowness of membrane processes, it is unlikely that any neuron could discriminate

such small temporal disparities in the arrival of two signals with certainty. It could suffice, however, if small temporal disparities in the arrival of an inhibitory and an excitatory input, for example, affected the activity of certain neurons probabilistically. Pooling of the activity of many such neurons could ultimately yield predictable responses.

Pre-pacemaker neurons are located at the top of a sensory hierarchy which determines the sign of D_t by evaluating a spatio-temporal pattern of modulations in phase differences and amplitudes^{9,12} (Fig. 4). Pre-pacemaker neurons exclusively innervate the pacemaker nucleus, which generates the EOD cycle, and modulate its frequency^{6,7}. Information about phase, that is, the timing of zero-crossings, is coded by T-type receptors which converge on higher-order neurons in the electrosensory lateral line lobe of the medulla. These neurons, in turn, converge on still higher-order neurons in the torus semicircularis in the midbrain, where differences in the timing of these spikes are evaluated⁹. Although this convergence progressively improves the temporal acuity of single neurons at each level, the smallest temporal disparity detected by the most sensitive neurons in the torus is only $10 \mu\text{s}$, a value which is still ten times higher than the behavioural threshold¹³. If the responses of such neurons are averaged over periods that are much longer than the latency

of the JAR (300 ms), a sensitivity to 1 μ s can be demonstrated. The sensitivity and the short latency of the JAR, therefore, require parallel convergence of large numbers of such toral neurons, carrying temporal information from wide areas of the body surface. This convergence ultimately occurs at the level of single neurons in the pre-pacemaker nucleus.

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Abnormal differentiation of thymocytes in mice treated with cyclosporin A

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Cyclosporin A (CsA) acts as a powerful immunosuppressive agent, and also, when given in repeated doses, can cause T-cell-dependent graft-versus-host disease and organ-specific autoimmune disease in rodents^{1–6}. This suggests that CsA interferes with the processes governing self-tolerance¹, either by nullifying the activity of T suppressor cells^{4,6} or by preventing the deletion of autoreactive T cells during ontogeny in the thymus². We report here that irradiated mice given repeated injections of CsA show striking dysfunction of the thymus. There are two different effects, the first of which is that CsA seems to block the differentiation of immature CD4⁺CD8⁺ thymocytes into mature CD4⁺CD8[−] and CD4[−]CD8⁺ cells expressing a high density of T-cell receptors and CD3 molecules. Second, CsA-treated mice show incomplete deletion of T cells expressing T-cell receptor molecules reactive to self H-2 I-E molecules.

Double positive cells comprise the bulk (~85%) of thymocytes and arise from a small (1–2%) subset of CD4[−]CD8[−] stem cells^{7–10}. The status of double positive cells is still unclear. A considerable proportion of these cells show surface expression of TCR/CD3 molecules but at a lower level than mature T cells^{11,12}. Most CD4⁺CD8⁺ cells seem to die soon after formation, but recent evidence suggests that some of these cells act as precursors of CD4⁺CD8[−] and CD4[−]CD8⁺ cells^{13,14}. The latter cells form about 15% of thymocytes and resemble mature T cells in expressing a high TCR/CD3 density. Although many CD4⁺CD8[−] and CD4[−]CD8⁺ thymocytes are exported from the thymus as mature T cells¹⁵, ~40% of CD4[−]CD8⁺ cells show little or no TCR/CD3 expression^{12,16}; these particular cells are

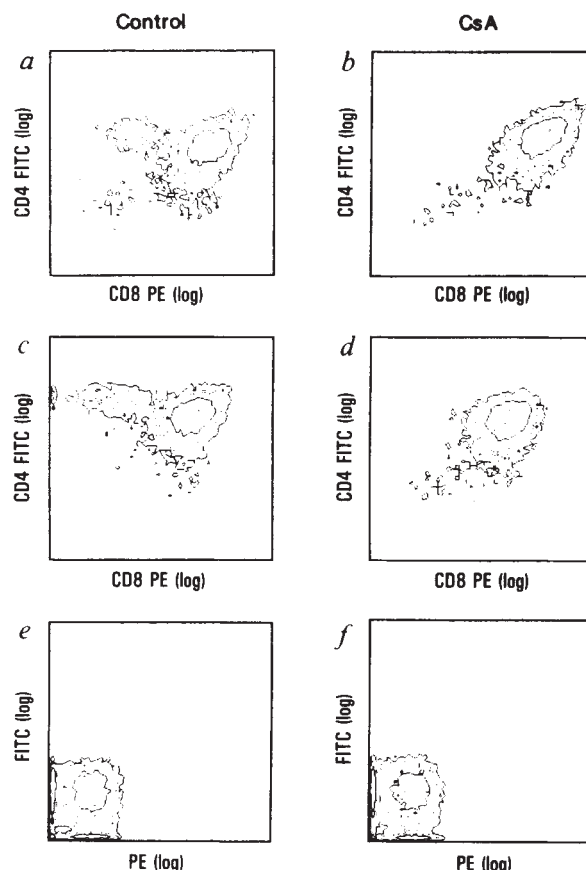


Fig. 1 Thymocytes were stained for CD4 versus CD8 expression as described in Table 1. *a, c*, Two control mice given 600 rad + anti-Thy 1 mAb and then olive oil daily for three weeks (*a*) or seven weeks (*c*). *b, d*, Two CsA-treated mice given 600 rad + anti-Thy 1 mAb and then CsA daily for three weeks (20 mg kg^{−1} day^{−1}) (*b*) or CsA daily for seven weeks (10 mg kg^{−1} day^{−1} for three weeks and then 20 mg kg^{−1} day^{−1} for the next four weeks) (*d*). *e, f*, Control staining for *c, d*, that is, staining with second reagents only. A discrete subset of CD4⁺CD8[−] cells is apparent in the control thymocytes (*a, c*) but not in the CsA-treated thymocytes (*b, d*).

viewed as an intermediate stage between CD4[−]CD8[−] and CD4⁺CD8⁺ cells¹⁶.

It is generally assumed that double positive cells reside predominantly in the cortex whereas CD4⁺CD8[−] cells and CD4[−]CD8⁺ cells are concentrated in the medulla^{7–10}. It is still not clear whether some of these single positive cells are also situated in the cortex. CsA has been useful in studying this problem because the thymus of rodents given a prolonged course of CsA injections shows selective atrophy of the medulla^{2,17–19}. The epithelial component of the medulla is destroyed^{18,19} and Ia (H-2 class II) expression is greatly reduced². In contrast the cortex of CsA-treated rodents is relatively intact and the thymus consists almost entirely of cortical tissue. Do suspensions of thymocytes from CsA-treated rodents therefore consist solely of cells with a 'cortical' phenotype?

To examine this question, 3–4-week-old (B6 × CBA/Ca)F₁ mice were exposed to sub-lethal irradiation (600 rad) and then given a three-week course of daily intraperitoneal (i.p.) injections of CsA (10 mg kg^{−1} day^{−1}) dissolved in olive oil; control irradiated mice received olive oil only. As shown in Table 1 cell suspensions prepared from the thymus of the control mice showed a near normal distribution of the four thymocyte subsets (78% CD4⁺CD8⁺, 4% CD4[−]CD8[−], 12% CD4⁺CD8[−] and 5% CD4[−]CD8⁺; 6% of thymocytes expressed detectable levels of

Table 1 Surface markers of thymocytes and LN cells from irradiated mice treated with CsA

Phenotype of thymocytes	Olive oil only (%)		Treatment for 3 weeks		Treatment for 7 weeks	
	Olive oil only (%)		Olive oil + CsA (10 mg kg ⁻¹) (%)	Olive oil + CsA (20 mg kg ⁻¹) (%)	Olive oil only (%)	Olive oil + CsA (20 mg kg ⁻¹) (%)
CD4 ⁺ CD8 ⁺	78.3		94.1	92.9	83.2	92.2
CD4 ⁺ CD8 ⁻	3.8		2.0	2.6	1.6	2.3
CD4 ⁺ CD8 ⁺	12.3		1.6	<0.1	9.8	1.0
CD4 ⁺ CD8 ⁺	5.1		2.1	3.7	2.7	1.7
V _β 8 ^{hi}	2.4		0.4	—	—	—
V _β 8 ^{lo}	3.5		4.7	—	—	—
CD3 ^{hi}	18.2		—	0.9	13.7	0.6
CD3 ^{lo}	13.9		—	19.4	14.2	9.1
V _β 11 ^{hi}	—		—	—	0.20	0.11
V _β 11 ^{lo}	—		—	—	0.82	0.77
Mean number of thymocytes per mouse	117.4 × 10 ⁶		42.2 × 10 ⁶	54.3 × 10 ⁶	94.1 × 10 ⁶	101.4 × 10 ⁶

Phenotype and number of LN cells	Olive oil only (%)		Treatment for 3 weeks		Total cells compared with control group (%)	Olive oil only (%)		Treatment for 7 weeks		Total cells compared with control group (%)
	Olive oil only (%)		Olive oil + CsA (20 mg kg ⁻¹) (%)	Total cells per mouse (×10 ⁻⁶)		Olive oil only (%)		Olive oil + CsA (20 mg kg ⁻¹) (%)	Total cells per mouse (×10 ⁻⁶)	
All LN cells	100	11.2	100	4.2	37.5	100	37.2	100	16.1	43.3
Thy 1 ⁺	67.0	7.5	16.0	0.7	8.9	75.0	28.0	31.3	5.1	17.9
CD4 ⁺	49.2	5.5	10.3	0.4	7.8	56.0	20.8	21.4	3.5	16.8
CD8 ⁺	19.4	2.2	5.1	0.2	9.7	20.3	7.6	9.4	1.5	19.7
CD3 ⁺	64.8	7.3	11.1	0.5	6.6	—	—	—	—	—
J11d ⁺	26.1	2.9	76.0	3.2	109.2	19.0	7.1	55.7	9.0	126.8

(B6×CBA/Ca)F₁ mice 3–4 weeks old (obtained from the Research Institute of Scripps Clinic, La Jolla) were exposed to 600 rad γ -irradiation²³ and given daily i.p. injections of CsA (Sandoz, Hanover, New Jersey) dissolved in sterile olive oil (50–100 μ l per mouse) or olive oil only until assay day²; antibiotics²³ were included in the drinking water. Two different batches of mice were prepared. The first batch of mice received CsA at a dose of 10 mg kg⁻¹ day⁻¹ for a three-week period. The second batch of mice was given a single i.p. dose of opsonizing anti-Thy 1 monoclonal antibody (mAb) (1 ml of 1:5 dilution of T24 ascites fluid²³) on the day of irradiation to destroy radioresistant T cells; pooled LN recovered from some of these mice four days later were very atrophic and essentially devoid (<1%) of Thy 1⁺ cells. The anti-Thy 1-treated mice were given CsA at a dose of 20 mg kg⁻¹ day⁻¹ for three weeks or 10 mg kg⁻¹ day⁻¹ for three weeks followed by 20 mg kg⁻¹ day⁻¹ from week 3 to week 7. The control mice for the two batches of mice received either 600 rad + olive oil (batch 1) or 600 rad + anti-Thy 1 mAb + olive oil (batch 2); the data for the two batches of control mice were indistinguishable and have been pooled for simplicity. Each mouse was assayed individually and the data shown in the table represents the mean values obtained for three mice per group (except for six mice for the controls shown in column 1 and two mice for the controls in column 4). Using standard techniques, single cell suspensions of thymocytes and LN cells (pooled axillary, inguinal and mesenteric LN) were incubated with unconjugated mAb specific for Thy 1.2 (J1j, rat IgM)²⁴, CD4 (GK1.5, rat IgG_{2b})²⁴, TCR V_β8 (8.1+8.2) (KJ16, rat IgG)¹¹ or TCR V_β11 (RR3-15, rat IgG) (see text); after washing, the cells were incubated with fluorescein isothiocyanate (FITC)-labelled mouse anti-rat IgG (Pel-Freez Biologicals, Rogers, Arkansas) followed by further washing. CD3 antigens were detected with a FITC-labelled anti-CD3 mAb (145-2C11, hamster)¹². For dual fluorescence, FITC-labelled cells were washed three times, incubated with biotinylated mAb specific for CD8 (3.168, rat IgM)²⁴, washed again and then stained with phycoerythrin (PE)-conjugated streptavidin (Biomed, Foster City, California). Stained cells (10⁴ cells per sample) were analysed by flow cytometry on a FACS IV (Becton Dickinson Immunocytometry Systems, Mountain View, California). The total yield of LN cells shown in the table refers to viable cells.

TCR V_β8 (8.1+8.2) molecules, the density of V_β8 expression being high (V_β8^{hi}) on about one-third of these cells (typical of mature peripheral T cells) and low-to-intermediate (V_β8^{lo}) on the remaining two-thirds of the cells. A very different pattern of staining was seen with thymocytes from CsA-treated mice. The vast majority (94%) of these cells consisted of double positive cells (Table 1). There was a slight reduction in CD4⁺CD8⁺ cells but a profound reduction of CD4⁺CD8⁻ cells. Only 1.6% of thymocytes were CD4⁺CD8⁻, that is, eightfold less than in control thymuses. There was also a marked (sixfold) reduction of V_β8^{hi} cells. Significantly, the proportion of V_β8^{lo} cells was not reduced.

A second batch of mice was prepared with a higher dose of CsA (20 mg kg⁻¹ day⁻¹). To destroy T cells surviving the conditioning dose of 600 rad, this batch of mice was treated with a single dose of anti-Thy 1 antibody on the day of irradiation (Table 1). When examined after three weeks, thymocytes from the CsA-treated mice again showed only a mild (<twofold) reduction of CD4⁺CD8⁺ cells relative to the controls. (For simplicity the data for the two groups of control mice have been pooled.) By contrast, CD4⁺CD8⁻ cells were virtually undetectable (<0.1%) (Table 1; Fig. 1b). To define mature T cells in the second batch of mice, thymocytes were stained for CD3 expression. Whereas thymuses from control mice contained 18% CD3^{hi} cells, these cells accounted for only 1% of thymocytes from CsA-treated mice. However, as for TCR V_β expression, the proportion of CD3^{lo} cells (19%) was not reduced relative

to the controls (14%). Essentially similar results were obtained for mice given CsA for seven weeks (Table 1; Fig. 1d): CD4⁺CD8⁻ cells were not entirely absent (1%), but the proportion of CD3^{hi} cells was very low (0.6%), that is, >20-fold lower than in the controls (Table 1). As at earlier time points, the proportion of CD4⁺CD8⁺ cells was only mildly reduced. Given the paucity of CD3^{hi} cells, however, many of the residual CD4⁺CD8⁺ cells in CsA-treated mice were presumably CD3^{lo} or CD3⁻, the phenotype reported for the precursors of double positive cells (see above).

Figure 2 shows double staining of thymocytes for CD3 versus either J11d or H-2 class I (K^bD^b) expression. The J11d antigen is expressed on ~90% of thymocytes but is not expressed on mature functional T cells²⁰; conversely, expression of H-2 class I molecules is high on mature T cells but low or absent on most (70%) thymocytes⁸. Whereas thymocytes from the control mice had a sizeable proportion of 'mature' T cells ~6% CD3^{hi} J11d⁻ cells (Fig. 2a, box 1) and ~10% CD3^{hi} class I^{hi} (Fig. 2c, box 2), these cells were very rare in CsA-treated thymuses (0.2% and 0.4%, respectively) (Fig. 2b, box 1; d, box 2).

The composition of lymph node (LN) cells from CsA-treated mice given anti-Thy 1 antibody *in vivo* is shown in Table 1 (bottom); groups of mice were assayed at three and seven weeks. There were three significant results. First, total numbers of Thy 1⁺ cells in LN from CsA-treated mice were 10-fold lower than in the controls at three weeks and fivefold lower at seven weeks. Second, the reduction of Thy 1⁺ cells was almost as

Table 2 $V_{\beta}11$ expression on $CD4^{+}$ cells from LN of I-E $^{+}$ irradiated mice treated with CsA for seven weeks

Mice tested	Treatment	I-E expression	Mouse number	% $CD4^{+}$ cells expressing: $V_{\beta}8$	% $CD4^{+}$ cells expressing: $V_{\beta}11$	% $CD8^{+}$ cells expressing: $V_{\beta}8$	% $CD8^{+}$ cells expressing: $V_{\beta}11$
B6	—	—	1	15.0	3.8	16.1	8.2
			2	16.8	4.3	16.7	7.4
			3	18.0	5.6	16.4	6.5
			mean:	16.6	4.5	16.4	7.4
(B6 $\times$ CBA/Ca) F_1	—	+	1	20.0	<0.1	24.9	2.1
			2	22.3	<0.1	26.6	3.2
			3	23.0	<0.1	26.7	2.0
			mean:	21.8	<0.1	26.1	2.4
(B6 $\times$ CBA/Ca) F_1	600 rad + CsA in olive oil	+	1	17.1	3.9	23.5	7.0
			2	20.1	3.2	19.2	7.3
			3	20.7	1.2	21.7	4.3
			mean:	19.3	2.8	21.5	6.2
(B6 $\times$ CBA/Ca) F_1	600 rad + olive oil only	+	1	17.0	<0.1	21.6	4.5
			2	18.7	<0.1	21.3	5.4
			mean:	17.9	<0.1	21.5	5.0

The LN suspensions from the mice in Table 1 (batch 2) were stained first with anti- $V_{\beta}8$, anti- $V_{\beta}11$ or anti- $CD4$ mAb followed by FITC-labelled mouse anti-rat IgG, and second with biotinylated anti- $CD8$ mAb followed by PE-labelled streptavidin. The cells were then analysed as described in Table 1. Note that cells were double-stained for V_{β} versus $CD8$ expression but not for V_{β} versus $CD4$ expression: the cells shown in the table as ' $CD4^{+}V_{\beta}^{+}$ ' were in fact $CD8^{+}V_{\beta}^{+}$. It is reasonable to equate $CD4^{+}V_{\beta}^{+}$ and $CD8^{+}V_{\beta}^{+}$ cells because $CD4^{+}CD8^{+}$ cells were virtually undetectable and because V_{β} expression on $CD8^{+}$ cells is restricted to $CD4^{+}$ cells (rather than B cells). It should be mentioned that studies with mice differing only at the *I-E* (*E α*) locus, B10.A(2R) (*kkkb*) and B10.A(4R) (*kkbb*) mice, have shown that levels of $CD4^{+}V_{\beta}11^{+}$ cells are high in I-E $^{-}$ 4R mice but very low in I-E $^{+}$ 2R mice (O.K. and E.-K.G., unpublished data). Although these data strongly suggest that $CD4^{+}V_{\beta}11^{+}$ cells are I-E reactive, $V_{\beta}11^{+}$ hybridomas paradoxically show very little overt I-E reactivity. The affinity of $CD4^{+}V_{\beta}11^{+}$ cells for I-E molecules thus appears to be quite low, that is, too low to cause triggering of mature T cells but sufficient to cause negative selection of early T cells in the thymus.

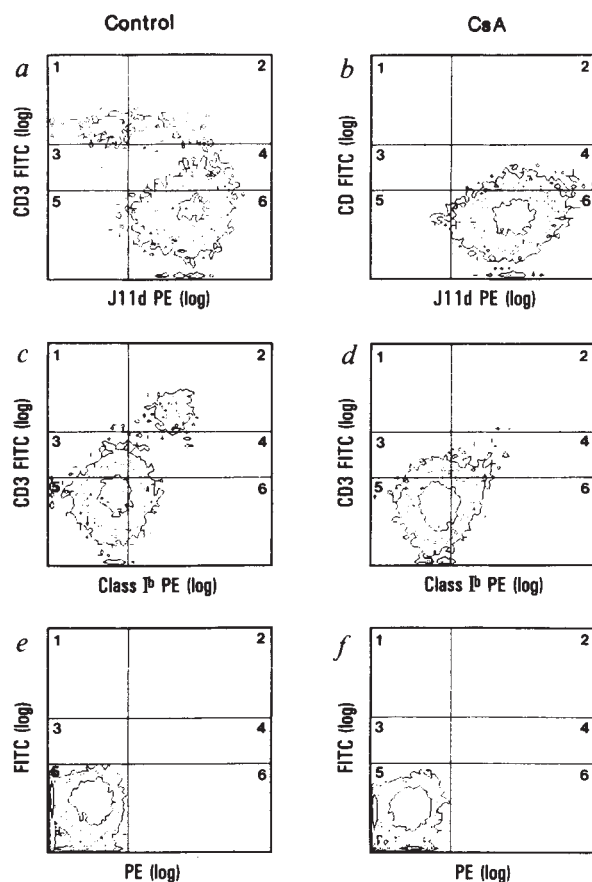


Fig. 2 Thymocytes were stained with FITC-labelled anti- $CD3$ mAb (see Table 1) versus biotinylated mAb specific for the J11d antigen (J11d, rat IgM) 20 or for H-2 class I (K^bD^b) (28-8-6s, mouse IgG $_{2a}$) 24 followed by PE-conjugated streptavidin (Table 1). The data shown are for thymocytes from a representative control mouse (600 rad + olive oil) (a, c, e) versus a mouse given CsA (10 mg kg $^{-1}$ day $^{-1}$ for three weeks and then 20 mg kg $^{-1}$ day $^{-1}$ thereafter) (b, d, f); both mice were tested at seven weeks after irradiation. The vertical axis has been divided into three compartments to enumerate $CD3^{+}$, $CD3^{lo}$ and $CD3^{hi}$ cells; the line to define class I lo against class I hi cells has been omitted for simplicity. For $CD3$ versus J11d staining (a, b), the control mouse has significant numbers of $CD3^{hi}J11d^{-}$ (box 1) and $CD3^{hi}J11d^{+}$ (box 2) cells; both of these subsets are very low in thymocytes from the CsA-treated mouse. The $CD3^{lo}$ thymocytes are nearly all J11d $^{+}$ (box 4); these cells are present in equivalent numbers in both the control and the CsA-treated thymocytes. For $CD3$ versus class I staining (c, d), the control mouse shows a discrete subset of $CD3^{hi}$ class I hi cells (box 2); this subset of cells is almost entirely absent in the CsA-treated thymocytes. The proportions of class I $^{-}$ and class I lo cells in the two groups are quite similar. e, f, Control staining (staining with the second reagents alone). A similar level of control (negative) staining was observed when the FITC-labelled anti- $CD3$ mAb was used to stain LN B cells.

marked for CD8⁺ cells as for CD4⁺ cells, and the ratio of these two subsets (1:2) was very similar to the control mice. [In contrast to the thymus, double positive cells were very rare in LN for both groups of mice (<1% of Thy 1⁺ cells).] Third, in contrast to T cells, CsA caused no reduction of B cells, that is, cells expressing the J11d antigen (which is expressed on nearly all LN B cells but not on LN T cells²⁰).

To study the induction of self-tolerance in CsA-treated mice, LN and thymocytes were tested for expression of V_β11 TCR molecules. Like V_β17a⁺ T cells²¹, mature V_β11⁺ T cells seem to be reactive to H-2 I-E molecules. Thus, these cells are expressed in I-E⁻ mice, for example B6, but are selectively deleted in I-E⁺ mice, for example (B6 × CBA/Ca)F₁ mice which were used in the above experiments (Table 2; and J. Bill, O.K. and E. Palmer, manuscript submitted); the deletion of V_β11⁺ T cells in I-E⁺ mice is near-complete for CD4⁺ cells but only partial for CD8⁺ cells. Significantly, in marked contrast to the control I-E⁻ mice, LN of CsA-treated I-E⁺ mice tested at seven weeks after reconstitution were not depleted of V_β11⁺CD4⁺ T cells (Table 2). In fact, 2.8% of CD4⁺ LN cells from these mice were V_β11⁺; this compared with 4.5% V_β11⁺ cells for the CD4⁺ subset from normal I-E⁻ B6 mice. For thymocytes, the proportion of V_β11^{hi} cells was very low in I-E⁺ mice although it is of interest that the ratio of V_β11^{hi} to CD3^{hi} thymocytes seemed to be higher in CsA-treated mice than in the I-E⁻ control group (Table 1, top).

These results indicate that CsA has two different effects on thymocyte development. First, CsA allows thymocytes to differentiate into CD4⁺CD8⁺ TCR/CD3^{lo} cells but prevents these cells from subsequently differentiating into mature TCR/CD3^{hi} thymocytes, that is, into subsets of CD4⁺CD8⁻ and CD4⁻CD8⁺ cells destined for export to the peripheral lymphoid organs. How does CsA block this transition? A considerable amount of evidence favours the notion that the production of mature T cells in the thymus depends on 'positive selection' of cells with specificity for 'self' H-2 molecules¹⁰: through their TCR molecules, a small proportion of early T cells (probably CD4⁺CD8⁺ cells) binds to H-2 determinants on cortical epithelial cells and these T cells receive a maturation signal. CsA might block this signal, either by interfering with lymphokine release²² or by some other mechanism. Alternatively, the maturation of thymocytes to TCR/CD3^{hi} cells might require the presence of medullary epithelial cells: by destroying medullary epithelium, CsA arrests thymocytes at the TCR/CD3^{lo} stage.

Second, in addition to suppressing positive selection (and/or T-cell maturation), the data on V_β11 expression indicate that CsA also impairs negative selection, that is, the deletion of autoreactive T cells. This result could explain the propensity for CsA-treated rodents to develop autoimmune disease after the end of CsA administration (although it is unclear whether V_β11⁺ T cells *per se* can mediate autoimmunity). The incomplete negative selection of T cells in CsA-treated mice might simply reflect reduced Ia expression in the atrophied thymic medulla². It is also conceivable, however, that CsA somehow impairs the delivery of 'tolerogenic signals', although there is no direct evidence for this. The possibility that CsA also interferes with the activity of T suppressor cells cannot be excluded.

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Note added in proof: Since submission of this paper, quite similar data have recently been reported by M. K. Jenkins, R. H. Schwartz and D. M. Pardoll (*Science* **241**, 1655-1658; 1988).

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Codon and amino-acid specificities of a transfer RNA are both converted by a single post-transcriptional modification

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An *Escherichia coli* isoleucine transfer RNA specific for the codon AUA (tRNA^{Ile}₂ or tRNA^{Ile}_{minor}) (ref. 1) has a novel modified nucleoside, lysidine (L; ref. 2) (Fig. 1a) in the first position of the anticodon (position 34), which is essential for the specific recognition of the codon AUA (ref. 1). We isolated the gene for tRNA^{Ile}₂ (ileX) and found that the anticodon is CAT, which is characteristic of the methionine tRNA gene. Replacement of L(34) of tRNA^{Ile}₂ molecule enzymatically with unmodified C(34) resulted in a marked reduction of the isoleucine-accepting activity and, surprisingly, in the appearance of methionine-accepting activity. Thus, both the codon and amino-acid specificity of this tRNA are converted by a single post-transcriptional modification of the first position of the anticodon during tRNA maturation.

The codon and amino-acid specificities of tRNA species are essential for accurate translation in protein biosynthesis. The codon specificity of tRNA is naturally defined by the nucleotide sequence in the anticodon; the amino-acid specificity of tRNA aminoacylation has however been a subject of much interest. In some cases, the amino-acid specificity is converted by substitution of a single base or several bases^{3-6,37}. Recently, the alanine specificity was found to depend critically on a single base pair G(3)-U(70) in the acceptor stem of *E. coli* tRNA^{Ala} (refs 7,8).

E. coli tRNA^{Ile}₂ recognizes only the isoleucine codon AUA¹. In the first position of the anticodon (position 34), this tRNA has a novel modified nucleoside, lysidine² (L; Fig. 1a), which seems from its structure to be derived from cytidine. In fact, in

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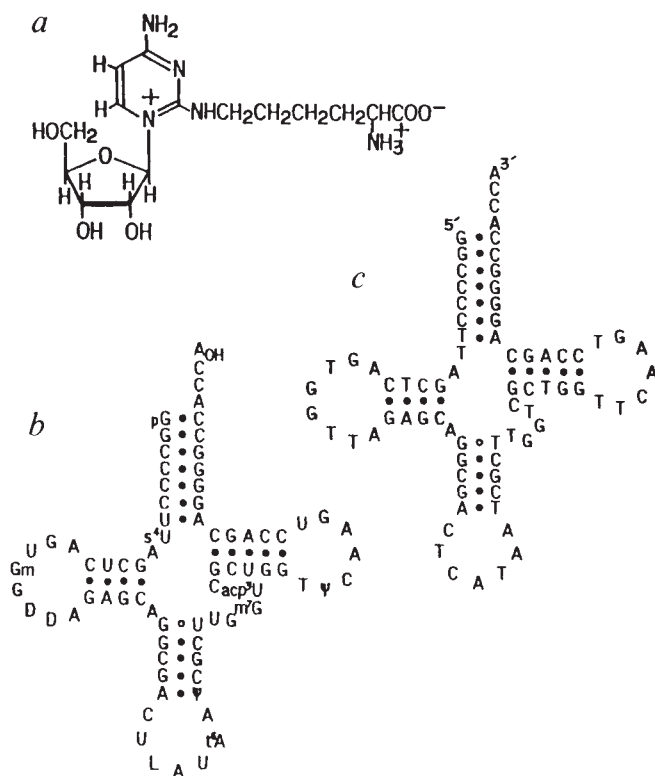


Fig. 1 Structures of *E. coli* tRNA^{Ile} and its gene. *a*, Chemical structure of Lysidine (L; ref. 2). *b*, Nucleotide sequence of *E. coli* tRNA^{Ile} (ref. 2). *c*, Nucleotide sequence of the structural gene for *E. coli* tRNA^{Ile} (*ileX*) in a clover-leaf model.

bacteriophage T4 and spinach chloroplast, the isoleucine tRNA specific to the codon AUA has an unidentified modified nucleoside in position 34 (refs 9,10), which the gene codes for as cytidine¹¹⁻¹³.

To determine the nucleoside precursor of lysidine, we cloned the gene for tRNA^{Ile} (*ileX*) from *E. coli* K12, using tRNA^{Ile} itself as a probe. A structural model for the gene (Fig. 1c; the flanking sequences will be reported separately), shows that this tRNA gene has the anticodon CAT. Thus lysidine derived from cytidine recognizes adenosine instead of guanosine in the third position of the codon. This is not wobble, but a unique case of complete conversion of base-pairing specificity. The codon specificity of the *E. coli ileX* gene product is converted from the methionine codon AUG to the isoleucine codon AUA by this single post-transcriptional modification of C(34) to L(34).

What difference in tRNA aminoacylation would there be, without this modification? If an unmodified tRNA species with the anticodon CAU accepted isoleucine, this should result in mistranslation of the codon AUG as isoleucine instead of methionine. We investigated this by aminoacylation experiments on a putative precursor of *E. coli* tRNA^{Ile}, in which L(34) was replaced by unmodified cytidine.

We prepared a tRNA species with the anticodon CAU—tRNA^{Ile}_{2(CAU)}—in which the nucleoside sequence was identical to that of tRNA^{Ile}_{2(LAU)} except for the first anticodon position (Fig. 2). The isoleucine- and methionine-accepting activities of this tRNA^{Ile}_{2(CAU)} were assayed (Fig. 3), and surprising differences from those of tRNA^{Ile}_{2(LAU)} were identified. The isoleucine-accepting activity of tRNA^{Ile}_{2(CAU)} was significantly reduced by replacement of L(34) by C(34); the initial aminoacylation velocity for tRNA^{Ile}_{2(CAU)} was less than 1/10 of that for tRNA^{Ile}_{2(LAU)} (Fig. 3a). This should prevent mistranslation of the codon AUG as isoleucine in place of methionine. Moreover, this tRNA^{Ile}_{2(CAU)} species had marked methionine-accepting activity: the initial velocity for tRNA^{Ile}_{2(CAU)} was more than 1/3 of that for tRNA^{Met}_m (Fig. 3b). Thus, a putative precursor of tRNA^{Ile}_{2(LAU)} (with the anti-

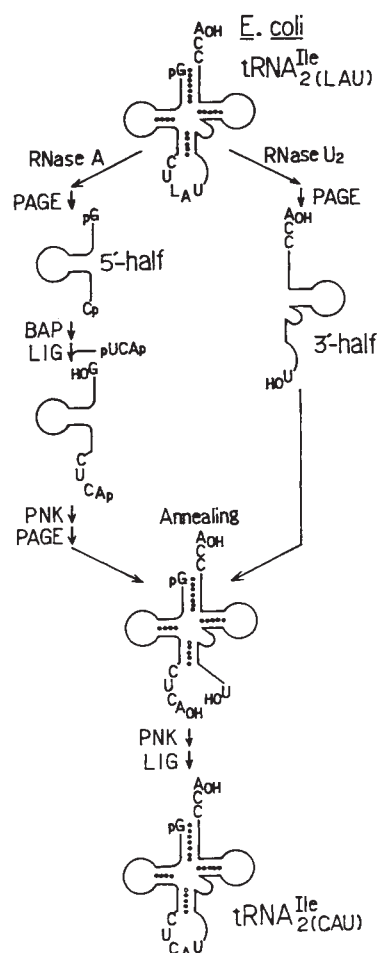


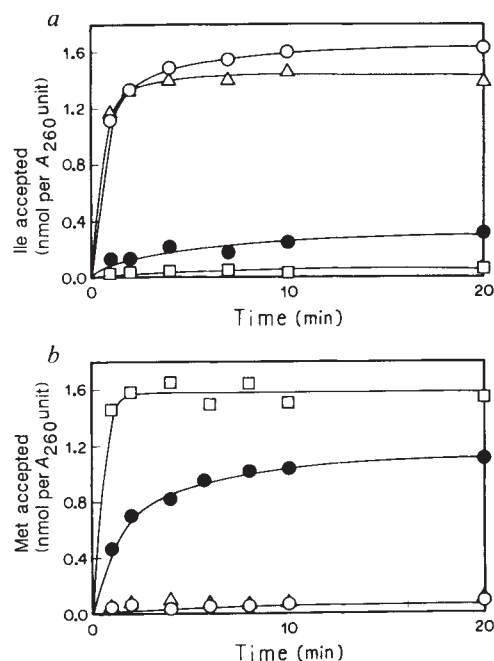
Fig. 2 Preparation of tRNA^{Ile}_{2(CAU)} 5' fragment. *E. coli* tRNA^{Ile}₂ (9 A₂₆₀ units) was purified by 7 M urea/10% polyacrylamide gel electrophoresis (PAGE) was partially digested with 20 µg of RNase A in 0.9 ml of 10 mM MgCl₂, 50 mM Tris-HCl (pH 7.6) at 0°C for 30 min, followed by phenol extraction and ethanol precipitation. From this digest, the 5' fragment (residues 1–32) was obtained by PAGE and dephosphorylated with *E. coli* alkaline phosphatase (BAP). HO-G-N₃₀-C-OH (0.9 A₂₆₀ units) was ligated with pU-C-Ap (0.9 A₂₆₀ units) using T4 RNA ligase (LIG), phosphorylated at the 5' terminus and dephosphorylated at the 3' terminus with T4 polynucleotide kinase (PNK). After purification by PAGE, the elongated 5' fragment (residues 1–35) pG-N₃₀-C-U-C-A-OH (0.40 A₂₆₀ units) was obtained. 3' fragment. *E. coli* tRNA^{Ile}₂ (5 A₂₆₀ units) was digested with 16 units of RNase U₂ in 50 mM CH₃COOK (pH 5.7) and 5 mM EDTA at 0°C for 30 min, followed by phenol extraction and ethanol precipitation. After purification by PAGE, the 3' fragment (residues 36–76) HO-U-N₃₇-C-C-A-OH (1.3 A₂₆₀ units) was obtained. Ligation. The elongated 5' fragment (1–35) pG-N₃₀-C-U-C-A-OH (0.38 A₂₆₀ units) and the 3' fragment (36–76) HO-U-N₃₇-C-C-A-OH (0.54 A₂₆₀ units) were mixed, annealed, phosphorylated at the 5' terminus of the 3' fragment with PNK, ligated with LIG in 15 mM MgCl₂, 3.5 mM dithiothreitol, 15 µg ml⁻¹ bovine serum albumin, 0.16 mM ATP, 50 mM HEPES-NaOH (pH 6.9) at 37°C for 30 min and then purified by PAGE to obtain pG-N₃₀-C-U-C-A-U-N₃₇-C-C-A-OH (residues 1–76), or tRNA^{Ile}_{2(CAU)}. All other techniques were as described³¹. The nucleotide sequence of this tRNA^{Ile}_{2(CAU)} was determined by the Donis-Keller method^{32,33} with 3' labelling using [5'-³²P]cytidine 3',5'-diphosphate³⁴.

codon CAU) may contribute to the translation of the codon AUG as methionine rather than isoleucine.

Why does methionyl-tRNA synthetase (MetRS) charge methionine to tRNA^{Ile}_{2(CAU)} and not to tRNA^{Ile}_{2(LAU)}? In *E. coli*, initiator methionine tRNA (tRNA^{Met}_i) has the anticodon CAU and the elongator tRNA^{Met}_m has the anticodon ac⁴CAU (ac⁴C,

Fig. 3 Time courses of isoleucylation (*a*) and methionylation (*b*) of tRNA^{Ile}_{2(LAU)} (○), tRNA^{Ile}_{2(CAU)} (●), tRNA^{Ile}_{1(GAU)} (△), and tRNA^{Met}_{m(ac⁴CAU)} (□) with *E. coli* synthetase.

Methods. All tRNA species were finally purified by 7 M urea/10% PAGE, dissolved in 20 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, and annealed by heating at 65°C for 10 min and cooling gradually to room temperature over 2–3 h. The isoleucine-accepting activity of each tRNA (0.1 A₂₆₀ units ml⁻¹, final) was measured in solution (0.5 ml) consisting of 100 mM Tris-HCl (pH 7.5), 2 mM ATP, 5 mM MgCl₂, 10 mM KCl, 45 μM [U-¹⁴C]isoleucine (28 mCi mmol⁻¹) and 16.7 μg ml⁻¹ (final) of *E. coli* isoleucyl tRNA synthetase³⁵. The methionine-accepting activity of each tRNA (0.04 A₂₆₀ units ml⁻¹, final) was measured in solution (0.5 ml) consisting of 100 mM Tris-HCl (pH 7.5), 2 mM ATP, 5 mM MgCl₂, 10 mM KCl, 60 μM [methyl-¹⁴C]methionine (60 mCi mmol⁻¹) and 21.5 mg ml⁻¹ (final) of *E. coli* S-100 fraction³⁶. These reaction mixtures were incubated at 37°C and at the indicated times, aliquots (50 μl) were removed and spotted on Whatman 2MM paper filter. The filter was washed three times with cold 5% CCl₃COOH, twice with ethanol, dried and its radioactivity counted in a liquid scintillation counter.



N⁴-acetyl-cytidine)¹⁴. The nucleoside in position 34 of tRNA species is known to be critical for recognition by MetRS; engineered species of tRNA^{Met} in which the original anticodon CAU (or ac⁴CAU) is replaced with UAU, AAU or GAU can no longer be aminoacylated by MetRS^{15,16}. A common feature of C and ac⁴C in position 34 thus constitutes a positive determinant for aminoacylation by MetRS. Furthermore, the sequence homology of tRNA^{Ile}₂ and tRNA^{Met}_m is as high as 70% (including modified nucleosides)¹⁴ and the tRNA^{Ile}_{2(CAU)} prepared here has the methionine anticodon CAU. Thus, tRNA^{Ile}_{2(CAU)} is efficiently aminoacylated by MetRS (Fig. 3b); the mature tRNA^{Ile}₂ is not (Fig. 3b). Interestingly, modification of cytidine to lysidine involves condensation of lysine to the pyrimidine ring of cytidine (Fig. 1a). This simple modification eliminates the positive determinant for the aminoacylation by MetRS, and thus the lethal misaminoacylation is avoided.

In contrast to MetRS, isoleucyl-tRNA synthetase (IleRS) does not seem to recognize the nucleoside in position 34, as the nucleosides in this position have totally different structures in tRNA^{Ile}_{1(GAU)} (ref. 14) and tRNA^{Ile}_{2(LAU)}, both of which are aminoacylated efficiently by IleRS. Surprisingly, we found that tRNA^{Ile}_{2(CAU)} was not effectively aminoacylated by IleRS (Fig. 3a). This indicates that IleRS does discriminate against tRNA species with the methionine anticodon CAU (or ac⁴CAU), thus possibly avoiding mistranslation of the codon AUG as isoleucine. If so, C(34) might constitute a 'negative determinant' for aminoacylation by IleRS.

A single post-transcriptional modification of C(34) to L(34) converts both the amino-acid specificity and the codon specificity of the *ileX* gene product during tRNA maturation. This suggests that, in evolution, the appearance of the modification converted a tRNA specific for AUG and methionine into a tRNA specific for AUA and isoleucine. During the establishment of the universal genetic code¹⁷, the simultaneous conversion of the two tRNA specificities might have contributed to the conversion of the methionine codon AUA into an isoleucine codon¹⁸.

In the mitochondria of some organisms (for example, mammals and *Saccharomyces cerevisiae*), the codon AUA is used for methionine^{19–25}, but in mitochondria of other organisms (for example starfish and *Schizosaccharomyces pombe*) this codon is still used for isoleucine^{26–30}. A deficiency in the isoleucine tRNA with lysidine might be responsible for the unusual cases where

the codon AUA is assigned to methionine instead of isoleucine; these cases do not seem to be related by evolutionary positions.

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Influence of an amino-acid residue on the optical properties and electron transfer dynamics of a photosynthetic reaction centre complex

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Bacteriopheophytin (Bphe) is the first kinetically and spectrally resolved electron carrier in the electron transfer pathway^{1,2} of the bacterial photosynthetic reaction centre (RC), the only membrane protein whose structure has been determined to atomic resolution³⁻⁵. The two Bpbes in the RC are not equivalent spectrally, and only the long-wavelength-absorbing Bphe, which is associated with the L subunit (Bphe_L)⁶, is active in electron transfer. There has been considerable speculation as to whether the spectroscopic red shift of Bphe_L and its exclusive involvement in electron transfer can be attributed to its interaction with a glutamic acid residue (L104) found within hydrogen-bonding distance to this Bphe (for examples see refs 7-10). Here we report the results of changing this glutamic acid residue to leucine, glutamine and lysine in RCs of *Rhodobacter capsulatus*. Low-temperature absorption spectra of these genetically modified RCs indicate that the glutamic acid residue at L104 is largely responsible for the spectroscopic red shift of the photoactive Bphe. However, time-resolved optical measurements indicate that the Glu^{L104} → Leu RC maintains the same high quantum yield of charge separation observed in wild-type by using only the electron transfer pathway associated with the L subunit. These results indicate that the Glu^{L104}-Bphe_L interaction is not the dominant contributor to the directionality of electron transfer in RCs.

The bacterial photosynthetic RC mediates extremely efficient electron transfer reactions that result in high quantum yields for the conversion of light to chemical energy^{1,2}. The crystal structure shows that the protein environment of the two Bpbes in the RC is quite different⁷. Glutamic acid L104 is within hydrogen-bonding distance of the C9 keto group (ring V) of the photoactive Bphe_L (Fig. 1), whereas no residues are in a position to hydrogen bond this group on the Bphe associated with the M subunit (Bphe_M). Resonance Raman⁸, infrared¹¹, and electron nuclear double resonance¹² spectral data all indicate that Bphe_L interacts with the glutamic acid residue at L104. This glutamic acid residue is phylogenetically conserved between the purple bacteria and is also found in many photosystem II RCs (as summarized in ref. 13). Glutamine is found in place of glutamic acid in the D1 polypeptide from *Anacystis nidulans*¹⁴ and in the L subunit of *Chloroflexus aurantiacus*¹⁵. We have replaced this glutamic acid residue with leucine, glutamine and lysine to determine whether substitution at this position affects the kinetics and directionality of electron transfer and/or the RC absorption spectrum.

Analysis of chromatophore membranes indicates that although substitution of glutamic acid L104 with lysine results in the loss of RCs from the membrane, both Glu^{L104} → Leu and Glu^{L104} → Gln mutants assemble RCs and are photosynthetically competent (Table 1). The near-infrared absorption spectra of the RCs isolated from these two mutants closely resemble that from the wild-type (data not shown). Low-temperature absorption spectra in the visible region of these genetically modified RCs (Fig. 1) confirm that the glutamic acid residue at L104 is

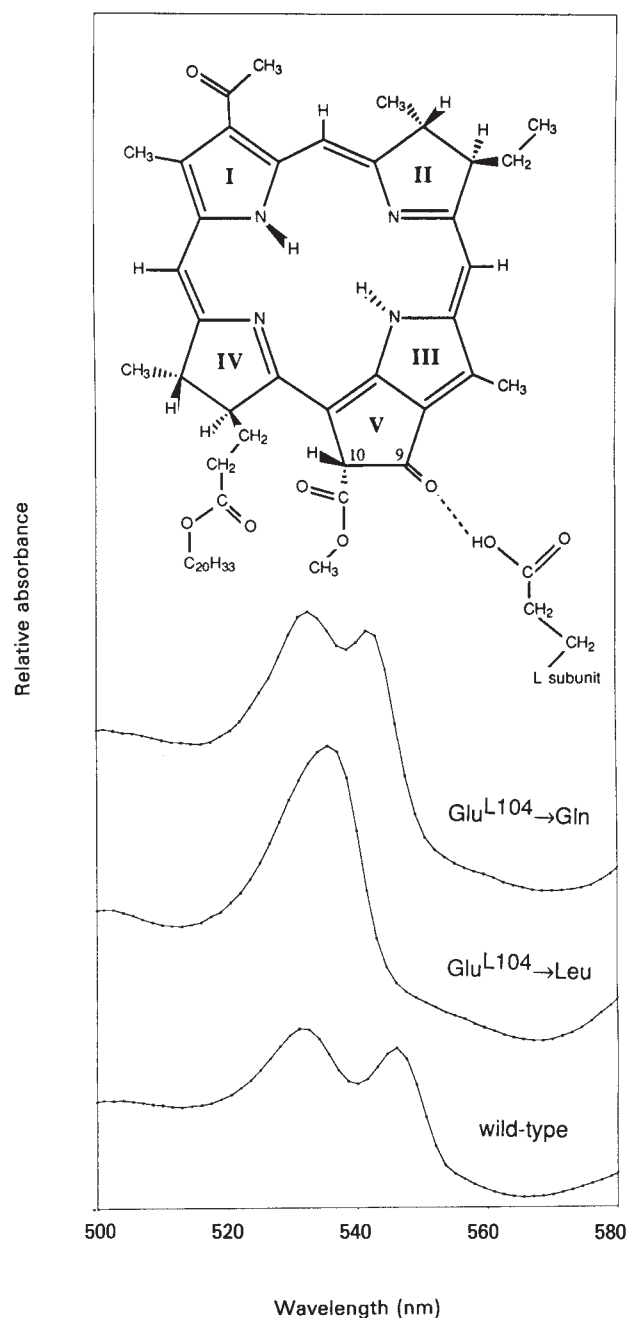


Fig. 1 Low-temperature (77 K) absorption spectra of purified RCs in 50% glycerol in the region of the Bphe Q_x transitions. In wild-type RCs, the Bphe_M band is at 531 nm and the Bphe_L band is at 546 nm. In Glu^{L104} → Gln RCs, the Bphe_M band is at 531 nm and the Bphe_L band is at 540 nm. In Glu^{L104} → Leu RCs, a single band is observed at 534 nm. These spectra were acquired with 1.5 nm resolution using a Lambda Diode Array Model 3840 spectrophotometer and Perkin-Elmer Model 7300 spectroscopic workstation. The inset shows a schematic diagram of the Bphe_L-glutamic acid interaction originally proposed by analysis of the *Rhodospseudomonas viridis* crystal structure⁷. X-ray structures have been determined for reaction centres from both *Rps. viridis*³ and *Rhodobacter sphaeroides*^{4,5}. Although the crystal structure of reaction centres from *Rb. capsulatus* has not been determined, extensive sequence similarities (see review in ref. 13) with *Rps. viridis* and *Rb. sphaeroides* suggest a homology in structures which facilitates mutagenesis experiments in *Rb. capsulatus*.

Table 1 Properties of RC mutations at glutamic acid L104

	Wild-type ²²	Glu ^{L104} → Gln	Glu ^{L104} → Leu
Nucleotide change		GAA → CAG	GAA → CTG
Photosynthetic growth	+	+	+
Electron transfer kinetics			
P* → P ⁺ Bphe _L ⁻ :			
P* emission decay (850–930 nm)	3.4 ± 0.5 ps	4.2 ± 0.6 ps	4.8 ± 0.6 ps
Bphe _L Q _X bleaching (530–540 nm)	3.4 ± 0.4 ps†	5.0 ± 0.8 ps	6.1 ± 1.0 ps
P ⁺ Bphe _L ⁻ → P ⁺ Q _A ⁻ :			
Bphe _L Q _X recovery (530–540 nm)	238 ± 35 ps†	265 ± 25 ps	270 ± 30 ps
Bphe _L anion decay (620–680 nm)	209 ± 16 ps	293 ± 35 ps	270 ± 30 ps

Mutagenesis procedures used have been described previously¹⁸. Mutagenized plasmids maintained in *E. coli* strain S17-1 (ref. 19) were conjugated into *Rb. capsulatus*. *Rb. capsulatus* deletion strain U43 (RC⁻LHI⁻LHII⁻) served as the background²⁰ for the determination of mutant phenotypes. The nucleotide sequences given in the table are of the mutagenized codon. The nucleotide changes in the L104 codon in Glu^{L104} → Lys are GAA → AAG. Photosynthetic growth assays were conducted as previously described¹⁸. RCs were purified with a modified¹⁸ DEAE chromatography procedure²¹. Electron transfer kinetics were measured by time-resolved absorption spectroscopy using a 350 fs excitation flash at 582 nm, as described elsewhere²².

† Note that the appearance and recovery of the Bphe_L bleaching in the wild-type reaction centres were measured in the Q_Y band (750–760 nm).

primarily responsible for the spectroscopic red shift of the photoactive Bphe. The Q_X band of Bphe_L moves closer to that of Bphe_M when glutamine is substituted at this position and cannot be resolved at 77 K from Bphe_M when a leucine residue is introduced. The single Bphe Q_X absorption band in the Glu^{L104} → Leu RC is slightly red-shifted relative to the wild-type Bphe_M band (534 nm versus 531 nm) which indicates that the Q_X bands of the two Bpbes are not degenerate. Other asymmetries in the protein environment of the two Bpbes may contribute to the remaining spectral differences of these pigments. These results indicate that hydrogen bond formation between ring V of the Bphe_L and a suitable residue at L104 makes the predominant contribution to the red-shifted absorption of Bphe_L compared with Bphe_M. The diminished red shift found in the glutamine mutation may be caused by weaker hydrogen-bonding interactions between Bphe_L and this residue.

Time-resolved optical measurements further show the spectral effects of a hydrogen-bonding residue at position L104, and demonstrate that both Glu^{L104} → Leu and Glu^{L104} → Gln RCs, like wild-type, only use the electron transfer pathway associated with the L subunit. Indeed, the electron transfer events in both mutants are only slightly slower than in wild-type (Table 1). For example, the decay of stimulated emission from P*, the excited primary electron donor, occurs with a time constant of 4–5 ps in the modified RCs, compared with approximately 3.5 ps in wild-type (Fig. 2) (P is a dimer of bacteriochlorophyll molecules). The rate of reduction of Bphe_L, which affords an additional measure of the initial electron transfer kinetics, can be assayed by the development of bleaching in this pigment's Q_X band and by the appearance of the Bphe anion band. The development of bleaching in the Q_X band of Bphe_L in the Glu^{L104} → Leu RCs is illustrated by the rapid kinetic phase in Fig. 3 ($\tau = 6.1 \pm 1.0$ ps). The wavelength of the bleaching in this modified RC (534 nm) is blue-shifted from wild-type (Fig. 3, inset), corroborating the differences seen in the ground-state absorption spectra (Fig. 1). The Q_X band bleaching in the Glu^{L104} → Gln RCs (data not shown), is also blue-shifted (536 nm) and develops with a somewhat slower rate compared with wild-type (Table 1). The kinetic traces in Fig. 3 for the Glu^{L104} → Leu RCs show a subsequent 270 ± 30 ps recovery of the Q_X band that accompanies movement of an electron from Bphe_L to Q_A. Again, as for the initial electron transfer reaction, the time constant for this second step in the charge separation process is only marginally slower in the mutants than in wild-type (about 270 ps versus 210 ps). In addition, for all three RCs the time constants for decay of P⁺Bphe_L⁻, measured by disappearance of the broad 620–680 nm Bphe_L band, agree with those determined by recovery of the Q_X band (Table 1). A spectral

effect of a hydrogen-bonding residue at position L104 is also apparent in a progressive blue shift of the Bphe_L band from 665 nm in wild-type to 655 nm in the Glu^{L104} → Gln RCs and to 640 nm in the Glu^{L104} → Leu RCs (data not shown).

The observation that the kinetics of the primary photochemistry in the Glu^{L104} → Leu and Glu^{L104} → Gln RCs are not significantly different from wild-type is but one indication that modifying residue L104 has not altered the quantum yield or directionality of charge separation. An additional compelling argument for a high quantum yield in the Glu^{L104} → Leu RCs can be made from Fig. 2, where it is seen that following a 350-fs flash, there is no decay of the bleaching of the 855 nm band of the primary donor (P) between 670 fs and 1.6 ns. This shows that the overall process: P* (670 fs spectrum) → P⁺Bphe_L⁻ → P⁺Q_A⁻ (1.6 ns spectrum) proceeds without any return of P* to the ground state (that is the yield of charge separation is essentially unity). This is precisely the same behaviour that is observed in wild-type and Glu^{L104} → Gln RCs. That Bphe_L, and not Bphe_M, is the pigment reduced by P* in both the Glu^{L104} → Leu and Glu^{L104} → Gln RCs is further supported by the observation that there is no evidence of any nanosecond (or slower) com-

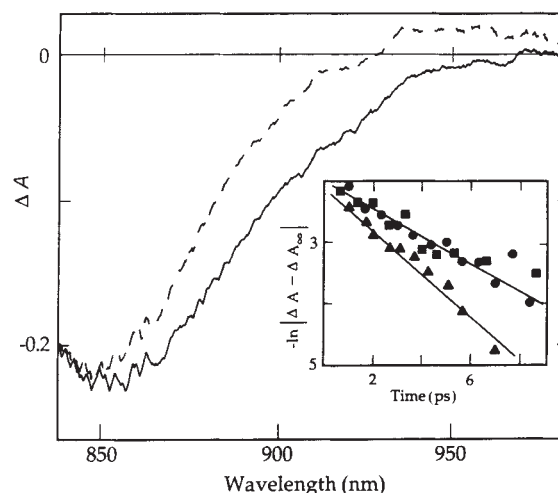


Fig. 2 Absorption difference spectra (ΔA) measured 670 fs (solid) and 1.6 ns (dashed) after a 350-fs flash in the Glu^{L104} → Leu RCs. The difference between these spectra in the 860–950 nm region reflects stimulated emission from P*. The inset is a log plot comparing the P* lifetime as measured by decay of the stimulated emission in Glu^{L104} → Leu (■), Glu^{L104} → Gln (●) and wild-type (▲) RCs. (The standard deviation in ΔA is ± 0.005 for these data.) The resulting time constants are listed in Table 1.

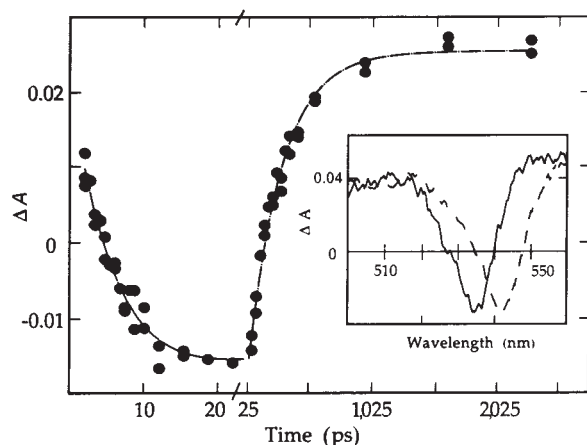


Fig. 3 Kinetics for the appearance and decay of bleaching in the 534 nm Q_X band of $Bphe_L$ observed after excitation of the $Glu^{L104} \rightarrow Leu$ RCs with a 350-fs flash at 582 nm. The solid line is a fit of the data to a constant plus two exponentials, yielding time constants of 6.1 ± 1.0 and 270 ± 30 ps. Similar results are obtained for the 536 nm bleaching in $Glu^{L104} \rightarrow Gln$ RCs (Table 1). The inset compares the position of the $Bphe_L$ bleaching observed at 12 ps in $Glu^{L104} \rightarrow Leu$ (solid) and the wild-type (dashed) reaction centres. By 2 ns, the bleaching has decayed completely to a positive absorbance associated with state P^+Q_A in both of these RCs, as well as in $Glu^{L104} \rightarrow Gln$ (see data points at long times in the main figure for examples).

ponent in the decay kinetics of the 620 to 680 nm $Bphe$ anion band, or of the $Bphe$ Q_X band bleaching between 530 and 540 nm, or of the primary donor bleaching at 855 nm. The presence of a nanosecond component would be expected if an electron was transferred to $Bphe_M$, reflecting either the charge recombination of P^+Bphe_M or a slow (because of the distance involved) electron transfer from $Bphe^-$ to Q_A (these RCs contain no secondary quinone, Q_B). A nanosecond or slower component would also be observed if the electron transfer from $Bphe^-$ to the primary quinone (Q_A) did not proceed with 100% yield. The absence of any such slow decay further demonstrates that these genetically modified RCs display primary photochemistry that is essentially the same as that found in wild-type RCs.

It is clear that there is an asymmetry in the environments of the two $Bphe$ s even after the substitution of leucine for glutamic acid, as demonstrated by the small red shift of the $Bphe_L$ Q_X band in the $Glu^{L104} \rightarrow Leu$ RC and the poor homology between the L and M subunits in the vicinity of the L104 residue¹⁶. In contrast, there is a high degree of homology between the D1 and D2 polypeptides in the vicinity of the analogous binding sites of the two pheophytins (Pheo) present in photosystem II RCs. The glutamic acid residue analogous to L104 is generally conserved in the D1 polypeptide of the photosystem II RC. Although the corresponding residue in the M subunit of the bacterial RC is a hydrophobic valine residue, the equivalent residue in the D2 polypeptide of the photosystem II RC is a glutamine¹³. Additionally, the increased environmental symmetry of the two Pheos is demonstrated in the absorption spectrum of photosystem II RCs, where the Q_X bands of the two Pheos are unresolved at 4 K (ref. 17). It seems reasonable that the principal source of the directionality of charge separation must be sought earlier in the electron transport chain, and/or in the combined effects of more than just a single pigment-protein interaction.

The glutamic acid residue at L104 in the RCs of *Rb. capsulatus* is principally responsible for the spectroscopic red shift of $Bphe_L$ compared with $Bphe_M$. Although mutations at this residue affect the electron transfer kinetics in the RC, Glu^{L104} is not the dominant contributor to the directionality of electron transfer, as the loss of the putative hydrogen-bonding interaction between $Bphe_L$ and Glu^{L104} has no significant effect on the ability of the

RC to carry out the primary electron transfer reactions resulting in P^+Q_A formation in high yield. Additional studies on $Glu^{L104} \rightarrow Leu$ and $Glu^{L104} \rightarrow Gln$ RCs and on other genetically modified RCs will provide a better understanding of the importance of pigment-protein interactions in the mechanisms of electron transfer in bacterial RCs. Our results show that an individual amino-acid residue can modulate the electronic properties of a chromophore, affecting both optical properties and electron transfer dynamics in pigment-protein complexes.

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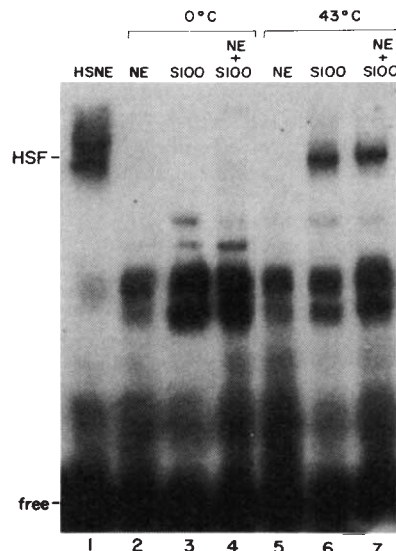
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Erratum

Activation *in vitro* of sequence-specific DNA binding by a human regulatory factor

Jeffrey S. Larson, Thomas J. Schuetz & Robert E. Kingston
Nature **335**, 372-375 (1988).

A correct version of Fig. 1 for this letter is shown below. In the original printing the image of the gels was laterally reversed.



Developments in neuronal cell culture

G. Banker and K. Goslin

The ability to grow neurons in culture has made possible great strides in the field of neuroscience. Advances in optical microscopy, together with techniques involving the retroviral transformation of neuronal precursors and cell fusion, will pave the way for further developments.

EIGHTY-ONE years ago, Ross Harrison described for the first time the development of neurons in culture. His landmark paper, which showed that nerve fibres arise as outgrowths from nerve cells, simultaneously provided the critical evidence necessary to establish the neuron doctrine and laid the foundation for the modern discipline of tissue culture. In the last twenty years, in parallel with an increasing emphasis on the cellular mechanisms underlying neuronal function, nerve cell culture has matured from a discipline practised by only a few to an indispensable and widely used tool of modern neurobiology.

Much of the progress made during this time has involved primary cultures of postmitotic neurons from embryonic or neonatal tissue, dissociated and allowed to differentiate in culture. It is now possible to culture neurons from virtually every region of the vertebrate nervous system, as well as from many invertebrates. Comparable progress has been made in the culture of glial cells, enhancing our understanding of their functions and importance; these studies are beyond the scope of this review.

The increased utility of nerve cell culture has arisen from many separate advances, and the insights that have resulted from attempts to improve nerve cell culture have also enhanced our understanding of the development of neurons *in situ*. Perhaps most important among these has been an understanding of the role of environmental factors in neuronal survival and development. In fact, cell culture, in combination with biochemical fractionation techniques, has become the standard assay for the identification of extracellular matrix constituents and trophic substances that govern the growth of fibres, and the maintenance of nerve cells and their connections^{1,2}.

Because even the smallest region of the brain contains many types of neurons, the ability to identify the cell types present in culture is essential. Fluorescence-activated cell sorting in combination with retrograde labelling has been used to pre-select specific cell populations³, while the increased availability of cell-type specific antibodies has made it feasible to identify cell types after introduction into culture. Finally, serum-free media have been developed which can be optimized for particular cell types.

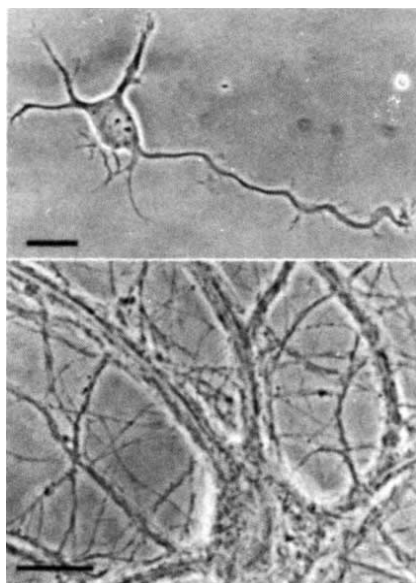


Fig. 1 Early in development, cultured neurons can be viewed in their entirety (above). Hippocampal neurons, like the one shown, have a single, long axon and several shorter processes that will become dendrites. After a few weeks in culture (below), the cell body and dendrites have increased markedly in size and are contacted by a dense network of axons from neighbouring cells. Scale bar: 8 μ m.

Until recently, the processes of cells in culture were usually termed "neurites" because their relationship to axons and dendrites was uncertain. It is now clear that many types of neurons develop both axons and dendrites in culture. They can be identified by specific markers such as MAP2 (for dendrites) and GAP-43 (for axons)⁴. Although confined to a 2-dimensional substrate, in most other respects these processes resemble their counterparts *in situ*⁵. In cultured hippocampal neurons (Fig. 1), the events that lead to the establishment of the axonal and dendritic domains are largely controlled endogenously, but early in their development processes have a surprising plasticity. After axotomy, a process that would otherwise have become a dendrite can become an axon and *vice versa*⁶. The development of peripheral neurons in culture is more obviously dependent on the environment; different conditions selectively enhance or inhibit the formation of dendrites⁷.

An enabling technology

Much of our knowledge concerning the dynamics of axonal growth and of growth

cone behaviour is derived from studies of neurons in culture. Many insights have been gained into the molecular basis for axonal elongation and into the interaction of growth cones with their environment. For example, contact with other fibres or application of neurotransmitters can rapidly inhibit the extension of growth cones⁸. Growth cones also release neurotransmitters, and contact with target cells can dramatically enhance this release within a matter of seconds⁹.

Tissue culture offers a new approach to the study of the cell biology of dendrites. Observations of dendritic growth and dendritic growth cones, of the dendritic transport of RNA and glycoproteins, and of the polarity orientation of the microtubules mediating dendritic transport have already begun^{4,10,11}.

The accessibility of cells in culture, and the ability rigorously to control their extracellular milieu, has made tissue culture a popular choice for biophysical studies. For example, the unique gating characteristics of the NMDA receptor, which may be responsible for triggering long-term potentiation (and under more extreme conditions, excitotoxicity) could hardly have been discovered without neuronal cultures¹². Using cell culture, the distribution of receptors on the dendrites of CNS neurons and their alteration by innervation have been mapped for the first time³. With the advent of patch recording, which requires that the neuronal surface be directly exposed, cell culture has become a technique of choice for the neurophysiologist.

Techniques of today

Recent advances in light microscopy will, within the next few years, provide fundamental new insights into the dynamic processes of living cells¹³. Video techniques and image processing, which enhance contrast, allow minute cellular structures to be seen clearly and their movements recorded. In addition, the intensification of faint fluorescent images has made it possible to observe fluorescently labelled molecules at the low light levels necessary to avoid cell damage. For example, by labelling living cells with fura-2, a Ca^{2+} -sensitive fluorescent dye, changes in the behaviour of growth cones have been correlated with changes in Ca^{2+} concentration¹⁴. In addition, fluorescent conjugates of known proteins can be introduced into

single cells and followed over time. This approach is beautifully exemplified by studies of the polymerization and depolymerization of individual microtubules in living cells following the microinjection of fluorescently labelled tubulin^{15,16}.

Despite the value of primary cultures, they cannot provide sufficient material for many biochemical studies and are difficult to manipulate using molecular genetic techniques. Clonal cell lines are an obvious alternative, but those presently available differ significantly from neurons *in situ*. Two new approaches provide better cell lines. The first involves the transduction of neuronal precursor cells with genetically engineered retroviruses containing immortalizing oncogenes¹⁷. When temperature-sensitive alleles of these oncogenes are used, proliferation can be blocked at non-permissive temperatures, permitting the development of a differentiated phenotype. The second involves fusion of an identified population of postmitotic neurons with cell lines that express some neuronal features¹⁸. The clonal cell lines that derive from the hybrids may express properties characteristic of the specific neuronal type used. If these techniques prove successful, nerve cell lines, like primary cultures, may ultimately become widely used.

Amid the widespread scientific and popular excitement that greeted the discovery of tissue culture — a *New York Times* headline in 1910 read "Build new tissue outside the body . . . a cancer cure" — Harrison cautioned that "in our enthusiasm for the novelty of the method we may forget the fundamental problems to the solution of which it may be able to contribute — if used critically"¹⁹. Perhaps even he would agree that nerve cell culture has finally come of age. □

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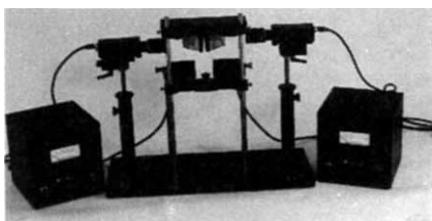
Gary Banker and Kimberly Goslin are at the Department of Anatomy, Albany Medical College, Albany, New York 12208, USA. For more information, fill in reader service number 100.

Neuroscientists in Toronto

Next week, learning and memory, psychotherapeutic drugs and the ion channels of neurons will be the talk of the town at the American Society for Neuroscience meeting in Toronto, Ontario. As a preview to the more than 175 exhibits, we feature products from a neuro-receptor ligand to a magnetometer for mapping the brain.

The Alzet **osmotic pump** from Alza Corporation in booths K103 and K105 is a miniature self-powered pump that continuously delivers test agents at closely controlled rates into mice, rats and other laboratory animals (*Reader Service No. 101*). The pump can be implanted subcutaneously or intraperitoneally to serve as a constant source of drug in experiments involving drug delivery. Alza's pump has no external connections, and does not require handling of the animal after surgical implantation. The pump consists of a collapsible reservoir of flexible, impermeable material, surrounded by a sealed layer containing an osmotic agent, and then by a semi-permeable membrane. When the filled pump is placed in an aqueous environment, the osmotic agent imbibes water at a rate that is controlled by the semipermeable membrane. The imbibed water generates hydrostatic pressure, which forces the pump's contents out at a constant rate. The Alzet pumps come in a variety of sizes, and come in packages of ten for \$99-\$223 (US).

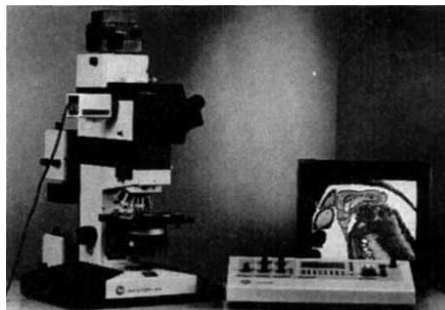
Stoelting Co., exhibiting in booths L118 and L120, has a wide-angle **eye movement tracker and television pupillometer** system that automatically eliminates shadows and adjusts for errors (*Reader Service No. 102*). Stoelting's instrument is based on the premise that pupil diameter variations are a more valid indicator of interest, arousal or stress than Galvanic skin response or heart rate. It simultaneously records pupil diameter with eye position, so the subject's response can be correlated with the object viewed. The camera that observes the eye is placed at a right angle to the subject's optical axis so it does not interfere with the subject's field of view. Stoelting says the system yields absolutely precise position analysis at any eye position because it uses pupil centre computation, not corneal reflected light tracking.



Stoelting's eye movement tracker and pupillometer system for interest, arousal or stress studies.

The \$13,500 (US) combined system comes with chin rest/camera mount, two nine-inch TV monitors, standard and infra-red TV cameras, eye illuminator and an analyser/computer.

The Multicon **video image analysis system** will be among the microscopy products on display in the Wild Leitz booth, number N101 (*Reader Service No. 103*). The \$17,200 (US) Multicon system has an analogue-controlled self-contained microprocessor which provides a range of



Nerve fibres can be outlined using one of the Leitz Multicon's special features.

contrasting effects for all microscopes equipped with a phototube, black and white television camera and a colour monitor. Special features include edge enhancement, pseudocolouring, contrast enhancement of specific areas of the image, and background subtraction. Fibres such as nerves can be outlined clearly using the Multicon's direction-dependent line intensification feature, says Leitz. Images also can be displayed in pseudo-3-dimensional mode, or be cleaned up by subtracting an averaged frame store image.

Neurochemicals

Kirkegaard & Perry Laboratories will be showing its HistoMark **immunohistochemical staining kits** for alkaline phosphatase or horseradish peroxidase reporter systems in booth L108 (*Reader Service No. 104*). The kits come in two colour combinations for each chemistry: red and blue for alkaline phosphatase, and orange and black for horseradish peroxidase. Enzyme activity is localized using a chromogenic dye, and contrast is enhanced by adding the nuclear counterstain. K & P says the HistoMark's high signal-to-noise ratio makes it useful for staining frozen and paraffin-embedded tissues, and other cellular preparations. Each \$95 (US) kit — sufficient for stain-



K & P's HistoMark immunohistochemical staining kits come with stain and counterstain.

ing 1,000 slides — includes chromogen and activator solutions, buffered substrate, counterstain and protocol.

Research Biochemicals Inc. now has chemically synthesized **D-myo-inositol-1,4,5-triphosphate** (IP_3), which it says is 99.9 per cent free of contaminating isomers (*Reader Service No. 105*). Most IP_3 is isolated from membranes, and is contaminated with the 2,4,5 isomer. RBI sells the IP_3 as a hexasodium salt, for \$275 (US) for 2 mg. The synthetic IP_3 is included in RBI's 1988 catalogue, available from booth H113.

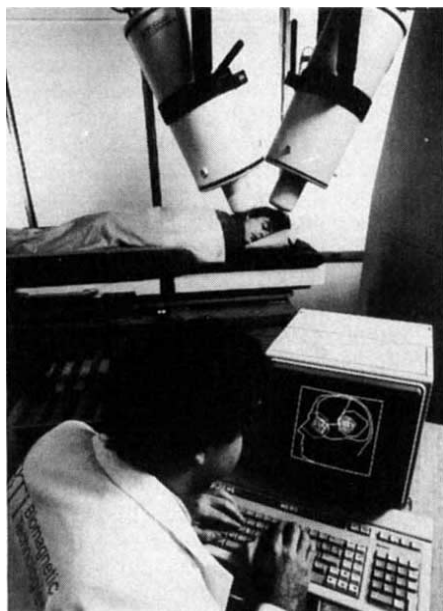
Five highly purified **gangliosides** are now available from Boehringer Mannheim Biochemicals, exhibiting in booths A100 and A102 (*Reader Service No. 106*). The list includes the monosialogangliosides GM1, GM2 and GM3; the disialoganglioside GD1a; and the trisialoganglioside GT1b. The gangliosides can be used as cell markers or potent antigens, and can be used in tumour research. Boehringer Mannheim says they are even pure enough to be used as standards for cell characterization, for the investigation of the mechanisms of cell-cell interactions in *in vitro* models, and in glycoconjugate research as substrates for glycosidases and receptors for glycoproteins.

DuPont's line of neurochemicals includes Quipazine, otherwise known as [piperazinyl- 3H]-, a selective radiolabelled ligand for the recently identified 5-HT $_3$ subclass of **serotonin receptors** (*Reader Service No. 107*). DuPont says Quipazine has a specific activity of 40–60 Cimmol $^{-1}$, affinity binding with a K_D of 1.3 nM, 50–70 per cent specific binding at 0.5 nM, and a binding site density (B_{max}) of 2.9 pmol g $^{-1}$ of tissue. The pharmacological profile of Quipazine corresponds to that of the 5-HT $_3$ receptor, says the company. The compound can be used to characterize the receptor, resolve the existence of various receptor sub-types, and identify and develop selective drugs. DuPont, exhibiting in booths H106, H108, and H110, sells a 250 μ Ci of Quipazine for \$525 (US).

Probing the brain

Biomagnetic Technologies' **Neuromagnetometer** can be used non-invasively

to measure brain activity and function in patients (*Reader Service No. 108*). The \$200,000 (US) Neuromagnetometer tracks the actual functional activity of the brain by detecting the magnetic fields created by the brain's electrical activity, making it applicable for the study of epilepsy, Alzheimer's disease, Parkinson's disease, substance abuse disorders, neuro-psychiatric disorders and spinal cord injuries. The Neuromagnetometer performs a



Biomagnetic Technologies' Neuromagnetometer maps brain activity by detecting magnetic fields.

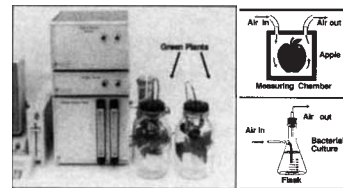
measurement called a magnetoencephalography (MEG), and is based on a superconducting quantum interference device (SQUID) — an ultra-sensitive magnetic field detector used in conjunction with cryogenic technology. It does not require the use of X-rays or radioactive isotopes, and allows the researcher to observe neuronal activities as they occur, in real time. Biomagnetic Technologies (on booth N104) says the Neuromagnetometer can detect messages 0.001 s in duration, and can pinpoint the location within 10–15 mm.

Bio-logic Systems Corp. will be displaying two major products in Toronto: its Brain Atlas **brain mapping systems** and the Bio-logic Banker, a storage device for **electroencephalograph (EEG) tracings** (*Reader Service No. 109*). Bio-logic's IBM computer-compatible Brain Atlas series are microprocessor-based systems for analysing EEG and evoked potential data to produce a topographic map of brain electrical activity. The Brain Atlas III has 32 channels, eliminating the need for interfacing with an EEG machine. The Brain Atlas EEG Mapper also processes 32 signal sources, but requires connection to an EEG machine. The Bio-logic Banker stores up to eight hours of EEG tracings — which on paper would weigh 77 pounds

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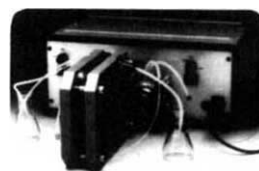
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— on a VHS cassette tape. For advanced investigations, it can be integrated with other Bio-logic products for frequency analyses, topographical brain mapping and statistical comparisons. Bio-logic is exhibiting in booth Q113.

Electrophysiology

Signal Processing Systems, exhibiting in booth F110, has a new system for discriminating action potentials from different neurons or motor units (*Reader Service No. 110*). The \$2,500 (US) SPS 8701 **waveform discriminator** uses a template-matching algorithm for on-line recognition of the characteristic waveforms of up to three different units. The system's special features include instantaneous frequency outputs for the three units being tracked, and the ability of the templates to adapt to changing waveforms. A built-in digital storage oscilloscope allows the discriminator to store incoming waveforms in memory, and to exploit the graphics capability of the computer to display them one-by-one for examination. Continuous data acquisition by direct memory access ensures that the whole waveform is visible regardless of the triggering threshold set, says the company. The SPS 8701 consists of a special-purpose printed-circuit board and operating software written entirely in machine code, and can be used with IBM-compatible computers.

Fine Science Tools, Inc. will be showing its **slice recording chamber** in booth F106 (*Reader Service No. 111*). The recording chamber can be used for electrophysiological, pharmacological and biochemical studies of tissue slices, and has a temperature-controlled bath and a humidified, oxygenated atmosphere. Slices may be superfused or submerged in media, according to the investigator's preference. Each \$1,195 (US) recording unit consists of a 2.5 cm-thick plexiglass recording head with two independent perfusion wells which hold 2.65 ml. Tissue slices are mounted on nylon mesh attached to removable plastic rings. The temperature control unit is sold separately, for \$895 (US).

The Isostim from World Precision Instruments is a complete **stimulator and isolator** in one package (*Reader Service No. 112*). WPI's Isostim can generate its own pulses or be driven by external command from a timer or computer. Pulse interval is continuously variable from 5 ms to 5.5 s, and pulse width is continuously variable from 50 μ s to 550 ms. In continuous mode, the Isostim generates continuous square waves, and in gated or external synchronized mode, externally applied pulses can generate pulse trains or single events. Single pulses may be pro-



The Isostim from World Precision Instruments.

duced using a front panel push-button, and the Isostim can be converted into a passive stimulus isolator using an external/DC mode. The Isostim is optically isolated from ground, and provides stimulus currents of up to 10 mA, with a maximum voltage range of 150 V. The output current is load-independent and the polarity is reversible. The \$895 (US) model A320D is powered by 9-V alkaline batteries, and the \$1,225 (US) model A320R comes with a rechargeable nickel-cadmium battery stack. WPI is exhibiting in booths K106 and K108.

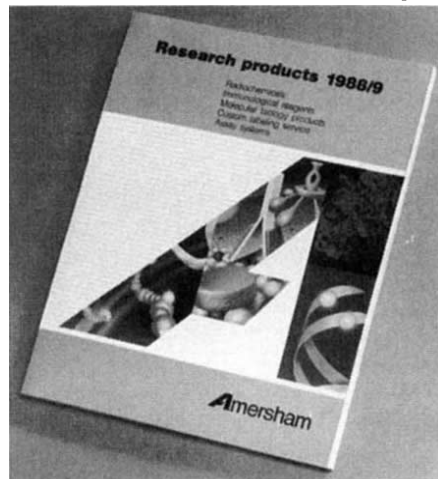
The literature shelf

Promega's 1988/1989 biological research products **catalogue and applications guide** is now available (*Reader Service No. 113*). The free catalogue contains 16 sections of comprehensive product information on Promega's line of molecular biological and immunological products. Promega will be displaying its research enzymes, nucleic acids, lambda DNA packaging systems, *in vitro* translation and transcription products, immunodetection systems for cDNA and Western blotting, cDNA synthesis systems, mapping systems, immunopurification products and electroporation instruments in booths N108 and N110.

Coulbourn Instruments, exhibiting in booths E101, E103, F100 and F102, has a new **physiology research brochure** which includes a selection guide for configuring systems for cardiovascular, skeletal, skin, gastrointestinal, central nervous system and pulmonary research applications (*Reader Service No. 114*). The free brochure features a variety of hardware including stimulators, telemetry systems, single-cell amplifiers, software, chart recorders and computer-based systems. It is meant to provide assistance in setting up experiments, from the selection of transducers to the options for recording.

Amersham's booths, numbers L101, L103, M100 and M102, are sure to have copies of its new **catalogue for life science research** (*Reader Service No. 115*). The 264-page catalogue lists over 2,000 products, including radiochemicals, a complete line of assay systems for *in vitro* diagnostic and research use, restriction and modifying enzymes, immunological reagents, liquid scintillation products.

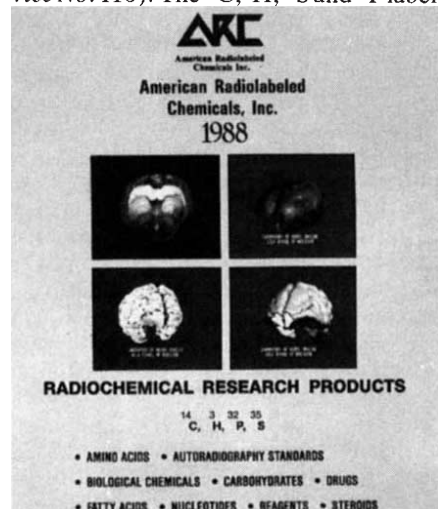
The free book also contains an expanded range of systems and kits for molecular biology, for performing cDNA synthesis, cloning, mutagenesis, DNA labelling, sequencing, translation and transcription. Over 250 new listings are included, plus



The new book on Amersham products.

specialized categories with application information, protocols, standards and comparison charts.

The 1988 catalogue from American Radiolabeled Chemicals, Inc. lists over 400 **radiolabelled chemicals** (*Reader Service No. 116*). The ^{14}C , ^3H , ^{35}S and ^{32}P label-



Includes over 400 radiolabelled chemicals.

led chemicals include amino acids, biological chemicals, carbohydrates, drugs, fatty acids, nucleotides, reagents and steroids. The catalogue also includes ^{14}C , ^3H , ^{35}S and ^{125}I labelled standards for autoradiography, which contain 14–16 pieces of plastic containing a wide range of radioactivity, mounted on microscope slides or coverslips. American Radiolabeled Chemicals is in booth N120. □

These notes are compiled by Carol Ezzell from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.